PERSISTENCE OF *PASTEURIA PENETRANS* AND ITS SUPPRESSION OF *MELOIDOGYNE ARENARIA*

By

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To My Grand Mom

Ayse Cetintas

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PERSISTENCE OF *PASTEURIA PENETRANS* AND ITS SUPPRESSION OF *MELOIDOGYNE ARENARIA*

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The persistence and suppressiveness of *Pasteuria penetrans* in field soil, and the effects of growing root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood race 1] non-host crops [bahiagrass (*Paspalum notatum* Flugge cv. Tifton 9) and rhizomal peanut (*Arachis glabrata* Benth. cv. Florigraze)] compared to weed fallow (including nematode susceptible weeds) were investigated. Soil from each treatment was tested after four (1999-2002) continuous year of growing peanut to determine its suppressiveness to *Meloidogyne arenaria* race 1. Over the course of the 4-year study, the average number of *P. penetrans* endospores per second-stage juvenile (J2), and percentage J2 with endospores attached, consistently increased. The average number of endospores attached to J2 increased to a high of 5.8 in the weed fallow plots by the autumn of 2001, but decreased in the autumn of 2002. Weed fallow plots sustained the highest percentage of J2 with endospores attached: 83% by the autumn of 2000. Rhizomal peanut and bahiagrass had lower percentages of J2 with endospores attached as compared with weed

fallow plots. Field studies for soil-depth distributions showed that *P. penetrans* endospores were recoverable at all depths and were as deep as 75 cm. However, in the laboratory most endospores remained in the top 0 to 25 cm. A soil suppressive test of soil taken from each treatment (bahiagrass, rhizomal peanut, and weed fallow) followed by four continuous year of peanut showed that soil contained suppressive levels of *P. penetrans* with the greatest in weed fallow plots. This indicates that for *P. penetrans* to remain at suppressive levels, some root-knot nematode infection and egg production must occur. In this study, autoclaving killed all biological agents (including *P. penetrans*), thereby allowing galling and egg mass formation. Air-dried and untreated soils contained antagonist (*P. penetrans*) that was suppressive to *M. arenaria* race 1. Biochemical analysis of *Meloidogyne* spp. females extracted from roots of peanut collected from the experimental site revealed the presence of *Meloidogyne arenaria* race 1 and *M. javanica* race 3 on peanut. Up to 29% of 290 individual females collected from peanut roots in the autumn of 2002 showed a typical esterase J3 phenotype for *M. javanica*.

CHAPTER 1
INTRODUCTION

Root-Knot Nematodes

Historical Background

Meloidogyne species, commonly named root-knot nematodes, are placed in the class Scernentea, order Tylenchida, family Meloidogynidae. This genus was first recognized in 1855 by Berkeley, who reported it to cause root-knot of cucumber in a greenhouse in England (Thorne, 1961). Muller, in 1884, described a species as *Heterodera radicicola* (Thorne, 1961). Throughout the years, studies revealed many physiological and biological differences among field populations of these nematodes (Christie, 1946; Christie and Albin, 1944). This led Chitwood to revise the genus in 1949, based on morphological differences. He recognized five species and one variety (Netscher and Sikora, 1990): *Meloidogyne arenaria* Neal, 1889, *M. exigua* Göldi, 1892, *M. hapla* Chitwood, 1949, *M. incognita* Kofoid and White, 1919, *M. incognita* var. *acrita*, and *M. javanica* Treub, 1885.

Former names reported in the literature and synonymized by Chitwood include *Anguillula marioni* Cornu, 1879, *A. arenaria* Neal, 1889, *A. vialae* Lavergne, 1901, *Heterodera javanica* Treub, 1885, *Tylenchus arenarius* Cobb, 1890, *Meloidogyne exigua* Göldi, 1887, *Oxyurus incognita* Kofoid and White, 1919, *Caconema radicicola* Cobb, 1924, and *Heterodera radicicola* (Greef, 1872) Muller, 1884. The latter was replaced by *Heterodera marioni* (Cornu, 1879) Marcinowski, 1909 (Thorne, 1961). Hirschmann

(1985) included 54 species in the genus *Meloidogyne*. Later Jepson (1987) reduced the number of species to 51. This was revised to include 60 species (Eisenback and Triantaphyllou, 1991). Recently, the updated number of described species of *Meloidogyne* spp. was reported as 80, 50% of which have been reported during the last 20 years (Karssen, 2002). The survey conducted by the scientists involved with The International *Meloidogyne* Project (IMP) in 75 countries reported that *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* were among the most common and economically important species of root-knot nematodes in agricultural soils (Netscher and Sikora, 1990).

Identification

Morphological characters. There is a great deal of similarity among the many *Meloidogyne* spp. that have been described, thereby making their identification difficult and confusing (Taylor and Sasser, 1978; Jepson, 1987). The morphology of the female, male, and second-stage juvenile (J2) is used in the identification of species of *Meloidogyne*. Key characters that are used for the female — morphology of perineal patterns, body shape and length, and shape and length of the stylet; male — shape of stylet cone, head, tail, spicules, head shape en-face view, and distance of the dorsal gland orifice (DGO) from the base of stylet knobs to the center of the orifice; J2 — length and shape of tail, head and stylet shape, and en-face view (Eisenback, 1985).

Cytogenetics. Cytology of root-knot nematodes is another important tool for their identification. A few *Meloidogyne* spp. reproduce by obligatory amphimixis (cross-fertilization) (Triantaphyllou, 1985). Most *Meloidogyne* spp., however, reproduce primarily by parthenogenesis. The parthenogenetic groups consist of obligatory

parthenogenetic (mitotic parthenogenetic) and facultative parthenogenetic (meiotic parthenogenetic) species. The latter is the most common method of reproduction among root-knot nematodes.

Some of root-knot nematodes species can be differentiated based on their chromosome numbers (Triantaphyllou, 1985). While differences in chromosome size and morphology have been detected among *Meloidogyne* spp., differences in chromosome numbers are most useful for identification purposes (Triantaphyllou, 1985). *M. arenaria* populations reproduce by mitotic parthenogenesis and have somatic chromosome numbers varying from 2n = 30 to 2n = 56.

Meloidogyne hapla, which has two cytological races (races A and B), is composed of facultative and obligatory parthenogenetic populations and amphimictic populations (Triantaphyllou, 1985). Most of the obligatory and facultative parthenogenetic populations have a haploid chromosome complement of 18 chromosomes. *M. hapla* race A, however, represents a unique case in that the chromosome number varies from n = 13 to n = 17. Also, *M. hapla* triploid populations have n = 28 and n = 34 chromosomes. Race B populations have a somatic chromosome number varying between 2n = 43 and 2n = 48, on the other hand, some have 2n = 30 to 2n = 32 chromosomes.

Populations of *M. incognita* reproduce entirely by mitotic parthenogenesis and occur as two cytological forms (Triantaphyllou, 1985). The triploid form, which has chromosome numbers varying from 3n = 40 to 3n = 46, is the most common and widely distributed around the world. The diploid form, which has chromosome numbers varying from 2n = 32 to 2n = 36, is less common (Triantaphyllou, 1985).

Meloidogyne javanica populations reproduce entirely by mitotic parthenogenesis and belong to one chromosomal form that may represent a triploid with a chromosome number varying from 2n = 43 to 2n = 48 (Triantaphyllou, 1985).

Host differentials. Differential hosts were first used in 1944 to distinguish species and intra-specific forms of root-knot nematodes (Christie and Albin, 1944). Currently, host tests are recommended to distinguish the four most common *Meloidogyne* spp., the four host races of *M. incognita*, and two host races of *M. arenaria* (Sasser, 1972). The four races of *M. incognita* are separated based on their reaction on tobacco (*Nicotiana tabacum* L. cv. NC-95) and cotton (*Gossypium hirsutum* L. cv. Deltapine 61). Race 1 does not infect either host, race 2 infects NC-95 tobacco, race 3 infects Deltapine 61 cotton, and race 4 infects both differential hosts (Sasser and Carter, 1982). Two races of *M. arenaria* are separated based on their reaction on peanut (*Arachis hypogaea* L. cv. Florunner). Race 1 infects Florunner peanut and race 2 does not. Three races of *M. javanica* have been proposed, although not widely recognized, based on their infection and reproduction on pepper (*Capsicum annuum* L. cv. Early California Wonder) and Florunner peanut (Rammah and Hirschmann, 1990). Race 1 does not infect either host, race 2 infects pepper, and race 3 infects peanut (Dickson, 1998).

Isozymes. Polyacrylamide gel electrophoresis (PAGE) has been used widely in studies of taxonomy, systematics, and population genetics and it has proved to be a very useful technique in the identification of species of *Meloidogyne* (Esbenshade and Triantaphyllou, 1985). Dickson et al. (1971) were the first to demonstrate some species-specific proteins that could be used in the separation of agriculturally important *Meloidogyne* spp. and some other plant-parasitic nematodes. Soon after, Hussey et al.

(1972) also drew attention to the importance of biochemical studies using enzyme phenotypes in nematode identification. Esterase, malate dehydrogenase, and α-glycerophospate dehydrogenase were useful in the identification of the four most common species of *Meloidogyne*, i. e. *M. incognita*, *M. arenaria*, *M. hapla*, and *M. javanica*. However, with the electrophoretic technology available during the mid-1960s these workers required mass homogenates of 100s to 1,000s of female *Meloidogyne* spp.

Soon thereafter electrophoretic technology changed so that isozyme patterns could be resolved from a single *Meloidogyne* female. Thus, esterase phenotypes became an even more accepted method in the identification of *Meloidogyne* spp. and drew considerable attention among researchers when an extensive effort was made to study many populations via The International *Meloidogyne* Project (Esbenshade and Triantaphyllou, 1990). Approximately 300 populations, originating from 65 countries, representing 16 different *Meloidogyne* spp., were studied with four different enzymes (Esbenshade and Triantaphyllou, 1985). Esterases, as in previous studies, were found to be the most useful in the identification of species. Approximately 94% of the populations of *M. hapla*, 98% of *M. incognita*, and 100% of *M. javanica* could be identified to species on the basis of esterase phenotypes alone. Two additional esterase phenotypes could identify *M. arenaria* with 98 to 100% accuracy. Esbenshade and Triantaphyllou (1985) showed that other isozymes also were useful for identifying *Meloidogyne* spp., especially when nematode species have similar esterase patterns. For example, *M. hapla* and *M. incognita* have a similar esterase patterns, but they can be distinguished easily by comparing their malate dehydrogenase (MDH) patterns.

Life cycle

Meloidogyne spp., like other plant-parasitic nematodes, have developmental stages that include an egg stage, four juvenile stages, and the adult stage (Eisenback and Triantaphyllou, 1991). Eggs are usually found in gelatinous matrices surrounding the posterior end of females. The eggs undergo embryogenesis, which culminates in a first-stage juvenile (J1). The J1 molts in the egg shell forming the second-stage juvenile (J2), which then hatches and migrates through the soil to locate a susceptible host (Eisenback and Triantaphyllou, 1991). This motile, vermiform infective stage penetrates host roots and establishes a permanent feeding site in vascular tissue (Williamson and Hussey, 1996). Due to nematode feeding, specialized feeding cells known as giant cells form in the vascular tissue. Giant cells are formed in peanut roots, pegs, and pods in the vicinity of the giant cells. The plant tissue increases in size (hypertrophy) and number (hyperplasia), thereby forming galls on roots, pegs, and pods. The nematode molts three more times, leading to the third and fourth juvenile stages and adults. These three molts occur in a relatively short period of the time, depending on the host plant and environmental conditions.

Root-knot nematodes display marked sexual dimorphism. The males are vermiform, whereas the females have a globose-pyriform or saccate shape. Females range in average length from about 0.44 to 1.30 mm and in average width from 0.325 to 0.700 mm. Males retain their vermiform shape and range in body length from 0.7 to 2 mm. The number of eggs laid in a gelatinous matrix is 200 to 1,500. Second-stage juveniles are the major survival stage in the absence of a host or in other unfavorable conditions.

Meloidogyne Species in Peanut

Distribution

The economically important root-knot nematode species associated with peanut production are *M. arenaria*, *M. hapla*, and *M. javanica*. These species infect peanut roots, pegs, and pods throughout the season, stunting the plants leading to a reduced yield (Dickson, 1998).

Meloidogyne arenaria, the peanut root-knot nematode, is recognized as the most widespread and destructive nematode pathogen of peanut (Porter et al., 1984). This nematode occurs from latitudes 40 °N to 33 °S (Taylor et al., 1982). The nematode is more of a problem in sandy soils and is seldom damaging in heavy clay soils. *M. arenaria* is known to occur in China, Egypt, India, Israel, the United States, and Zimbabwe (Ibrahim and El-Saedy 1976; Zhang, 1985; Minton and Baujard, 1990). In the United States, race 1 is common in peanut fields in Alabama, Arkansas, Florida, Georgia, and Texas, while patchy occurrences have been reported in North Carolina, South Carolina, and Virginia. Numerous juveniles are generally distributed throughout the soil profile of infested fields. In deep, sandy soils the largest numbers of juveniles are found 30 to 120 cm deep during planting time (Dickson and Hewlett, 1988). *M. arenaria* causes substantial yield losses even at relatively low population densities. For example, initial population densities of 9 to 19 J2/100 cm^3 of soil caused 10% suppression of yields in Texas (Wheeler and Starr, 1987), whereas the damage threshold in Florida can be as low as one J2/100 cm^3 of soil (McSorley et al., 1992).

Meloidogyne hapla (Chitwood, 1949), northern root-knot nematode, occurs in the northern hemisphere and cooler regions of the world, primarily between latitudes 34 °N

and 43 °N (Eisenback and Triantaphyllou, 1991). *M. hapla* has been reported in all peanut producing states in the USA with the exception of Florida (Dickson, 1998).

Meloidogyne javanica (Treub) Chitwood, the Javanese root-knot nematode, has an extensive host range and occurs between latitudes 33 °N to 33 °S. This nematode was detected for the first time on peanut in Zimbabwe (Martin, 1958). Later, other reports were made in Africa (Ibrahim and El-Saedy, 1976; Rammah and Hirschmann, 1990; Tomaszewski et al., 1994), India (Patel et al., 1988; Sakhuja and Sethi, 1985), and Brazil (Lordello and Gerin, 1981). Minton et al. (1969) reported *M. javanica* on peanut in Georgia for the first time in the United States. Later, the nematode was reported in Texas (Tomaszewski et al., 1994), and just recently, *M. javanica* was reported on peanut in Levy County, Florida (Lima et al., 2002; Cetintas et al., 2003).

Symptoms

Peanut plants that are infected with the peanut root-knot nematode generally have noticeable above and below-ground symptoms. In most cases, visible symptoms normally appear 45 to 75 days after planting, but the most severe symptoms may become visible after 90 to 120 days. This depends on the initial population density of nematodes in the soil and on the environmental conditions present 100 to 135 days after planting (Dickson, 1998; Minton and Baujard, 1990). Under hot dry conditions the nematode can weaken and kill plants. Root-knot nematodes on peanut are patchy in distribution, and infected plants have characteristic symptoms. Heavily infected plants are often severely stunted, became chlorotic, and have reduced numbers of pegs and pods.

Root-knot can be diagnosed by examining the roots, pegs, and pods for the presence of female nematodes (Dickson, 1998; Minton and Baujard, 1990; Porter et al., 1984). The females, which are pearly white, can be dissected from galled tissue.

Second-stage juveniles infect peanut roots soon after planting. One to 2 days following penetration by J2 abnormal swelling starts to develop. However, the formation of galls and egg masses does not really become apparent on the roots until approximately 55 to 90 days after planting, depending on the number of nematodes present in the root tissue (Dickson, 1998). Nematode galls can be distinguished easily from rhizobium (*Bradyrhizobium*, nitrogen fixing bacteria) nodules because they have a different shape and structure. Rhizobium nodules are distinct, round swellings on the roots, which can be detached easily. In contrast, nematode galls are irregularly shaped swellings that cannot be detached without destroying the integrity of the root. Rhizobium nodules also may be infected by nematodes, and they can serve as infection and reproduction niches for the peanut root-knot nematode (Minton and Baujard, 1990; Porter et al., 1984). The detection of galls on peanut roots is more difficult than on many other hosts such as tomato, tobacco, and soybean. The galls on peanut roots are small and generally discrete, whereas galls on other host crops, such as tomato, squash, and tobacco, may be large and coalesced.

Root-knot nematodes may infect pegs and pods soon after the initiation of pod set, which begins about 45 days after planting. Early infection can lead to a weakened peg and an aborted pod. Many infected pegs and pods may fall free from the plant at harvest. Late in the season, heavily infected plants result in substantial yield losses. Furthermore,

nematode-galled tissue is vulnerable to infection by soilborne fungi, which may lead to root deterioration (Dickson, 1998).

Management

Chemical control, crop resistance, crop rotation, other cultural practices such as fallowing, and biological control are among some of the management strategies that have been suggested for management of root-knot nematodes (Barker et al., 1998). Chemical control is considered the most effective and reliable method for controlling nematodes in peanut production (Kinloch, 2001). Fumigants and nonfumigants are two types of chemicals commonly used. Of the fumigants currently available, 1,3-D (1,3-dicloropropene) or Telone II (Dow AgroSciences, Indianapolis, IN) has been found to be the most effective nematicide in terms of consistency and provide yield increases in peanut (Kinloch and Dickson, 1991). In addition, several nonfumigants are labeled for use on peanut. The nonfumigant, aldicarb (2-methyl-2 (methylthio) propionaldehyde 0-(methylcarbamoyl) oxime or Temik 15G (Bayer CropScience, Research Triangle Park, NC) has been shown to be effective as an at-peg initiation treatment for managing nematodes in peanut. A broadcast application of Telone II followed by an application of Temik 15G at-peg initiation may be the best option in heavy root-knot nematode infested fields (Kinloch, 2001). Another nonfumigant, fenamiphos (ethyl 3-methyl-4-(methylthio) phenyl (1-methylethyl) phosphoramidate) or Nemacur 15G (Bayer CropScience, Research Triangle Park, NC), could be another good choice for suppressing nematode populations in peanut production fields.

One of the developing methods for root-knot nematode management in peanut is resistant cultivars. Recently two peanut cultivar lines, Coan (Simpson and Starr, 2001)

and Nematam (Simpson et al., 2003), were released with root-knot nematode resistance. Coan was phased out because Nematam was bred as a replacement cultivar. Neither of these cultivars has gained widespread adoption but research is under way to develop genotypes with root-knot nematode resistance with satisfactory agronomic characteristics.

Cultural practices are another important root-knot nematode management program that peanut producers rely on. Crop rotation with cotton, corn, and grasses such as bahiagrass (*Paspalum notatum*), bermudagrass (*Cynodon dactylon*), and sorghum (*Sorghum bicolor*), are some crops used in crop rotation for reducing root-knot disease in peanut. Despite allowing some root-knot nematode reproduction, corn is considerably less susceptible than peanut and is generally effective in the reduction of root-knot nematode population densities. Cotton is a very successful rotation crop because it is not a host for the peanut root-knot nematode nor is peanut a host for the cotton root-knot nematode. The latter is highly problematic on cotton in the southeastern USA. A two-year rotation of peanut and cotton is effective in managing the root-knot nematode problems on both crops. In general, however, after growing peanut, a reliable option is to rotate to a different crop for three or more years to reduce population densities of root-knot nematodes (Kinloch, 2001). Crop rotation schemes are most effective when the alternate crops are maintained weed free. Leaving fields fallow also is a method that could be used to manage the peanut root-knot nematode, but this tactic has very limited use because of wind and water erosion. Hairy indigo, Alyce clover, and morning glory are some important peanut root-knot nematode susceptible weeds that are common in

peanut fields. Similarly, volunteer peanut should not be allowed to grow in either fallow fields or in alternate crops in crop rotation schemes (Kinloch, 2001).

Biological control of nematodes is defined as a reduction of nematodes by the action of living organisms (other than nematode-resistant plants) that occur naturally (Stirling, 1991). Biological control is one of the promising non-chemical methods to manage root-knot nematodes. Recently, *Pasteuria penetrans* (Thorne) Sayre & Starr has been recognized as a potential biological control agent for *M. arenaria* race 1 in peanut fields in Florida and Georgia (Dickson et al., 1994; Minton and Sayre, 1989). *P. penetrans*, an endospore-forming bacterial obligate parasite of nematodes, is reported to cause soils to become suppressive to the peanut root-knot nematode (Dickson et al., 1994).

Pasteuria Species

Field observations (Dickson et al., 1994; Minton and Sayre, 1989), and greenhouse and microplot experiments (Brown and Smart, 1985; Channer and Gowen, 1988; Chen et al., 1996; Davies et al., 1988; Oostendorp et al., 1991) have shown that *Pasteuria* can aid in the control of plant-parasitic nematodes. *Pasteuria* spp. have been reported on various nematode hosts (323 nematode species) and in many different environments throughout the world (Atibalentja et al., 2000; Chen and Dickson, 1998).

Historical Background and Taxonomic Status

Pasteuria represents a genus of gram-positive mycelia endospore-forming bacteria that parasitizes nematodes and cladoceran *Daphnia* (Sayre and Star, 1988). Metchnikoff (1888) was the first to describe *Pasteuria* as an internal bacterial parasite of *Daphnia magna* Straus (water fleas). He named the bacterium *Pasteuria ramosa* and unsuccessfully tried to culture it (Sayre, 1993). The first report of an organism

resembling *Pasteuria* in a nematode was by Cobb (1906). He concluded this organism was an internal parasite of nematodes, and mistakenly described it as "perhaps monads" of a parasitic sporozoan. This error of naming the organism as a protozoan was to persist for nearly 70 years. A more precise, but still incorrect, placement was suggested by Micoletzky (1925), who found a nematode parasite having spores similar to those reported by Cobb. Micoletzky proposed that these spores belong to the genus *Duboscqia* Perez, 1908, again a sporozoan group. Later, Thorne (1940) described a parasite from *Pratylenchus pratensis* (de Man) Filipjev and considered it to be similar to the nematode parasite described by Micoletzky. The parasite was named *Duboscqia penetrans*. Mankau (1975) studied this organism with an electron microscope thereby revealing its bacterial nature. However, Mankau erroneously placed the organism in the *Bacillus* genus, naming it *Bacillus penetrans*. Sayre and Starr recognized that the bacterium more closely resembled the actinomycete *P. ramosa* (Sayre et al., 1983), and subsequently renamed the organism *Pasteuria penetrans* (Sayre and Starr, 1985). This classification was accepted by Bergey's Manual (Sayre and Starr, 1989).

Morphology and Development

Members of *Pasteuria* are obligate parasites that begin their development as dichotomously branched septate mycelia (Sayre and Starr, 1985; 1988; Williams et al., 1994). The terminal hyphae of the mycelia increase in size to form sporangia and eventually individual endospores. Mycelial colonies are irregular in shape and resemble cauliflower florets or elongated grapes when observed with an electron microscope. The primary colonies are formed by fragmentation of mother colonies, and the daughter colonies develop into doublets and quartets of sporangia, and finally the single

sporangium develops into a mature endospore. Mature endospores are nonmotile, spherical in shape, and resistant to desiccation, chemical lysis, and elevated temperatures (Williams et al., 1994). *Pasteuria* spp. differ from each other in host specificity, morphology, and developmental characters, such as the size and shape of sporangia and endospores (Sayre and Starr, 1989). No *Pasteuria* has been grown in pure culture; they are attainable from a nematode or a water flea host.

The genus *Pasteuria* currently contains four described species, three of which are parasitic on nematodes *P. thornei, P. nishizawae*, and *P. penetrans* and one is on the water flea, *P. ramosa*. *P. ramosa*, the type species, has never been observed to parasitize nematodes. It is parasitic on aquatic organisms in the genera *Daphnia* and *Moina* (water fleas) (Ebert et al., 1996; Sayre et al., 1983). *P. thornei* (Sayre and Starr, 1988; Starr and Sayre, 1988) infects lesion nematodes belonging to the genus *Pratylenchus*. *Pasteuria nishizawae* is found on cyst nematodes of the genera *Heterodera* and *Globodera* (Sayre and Starr, 1989, Sayre et al., 1991), and *P. penetrans* parasitizes root-knot nematodes belonging to the genus *Meloidogyne* (Starr and Sayre 1985; Starr and Sayre, 1988).

Various groups of *P. penetrans* are classified by their ability to attach selectively to different species of nematodes. These groups vary in morphological characters such as the shape and size of endospores (Bird et al., 1990; Williams et al., 1994). In recent years, at least three different groups of *Pasteuria* have been isolated from different hosts, and each has been proposed as a new species. These newly proposed *Pasteuria* species include *Heterodera goettingiana* (cyst pea nematode) Liebscher in Munster, Germany (Sturhan et al., 1994), *Belonolaimus longicaudatus* Rau (sting nematode) in Florida, USA (Giblin-Davis et al., 1995), and *Heterodera glycines*, in Illinois, USA (Atibalenji et al.,

1998). A newly proposed *Pasteuria* species from *Belonolaimus longicaudatus* Rau (sting nematode) in Florida, USA was designated as '*Candidatus Pasteuria* usgae' sp. nov. (novel taxon). This designation was based on its taxonomically relevant characteristics such as morphology, morphometrics, host range, and 16S ribosomal gene (rRNA) sequence similarities (≥93%) to the other species and strains of *Pasteuria* from nematodes and water fleas (Giblin-Davis et al., 2003). The phylogenetic analysis with 16S rRNA suggests this species is most closely related to *P. penetrans* from *Meloidogyne* spp. and the *Pasteuria* strain recently isolated from the soybean cyst nematode (*Heterodera glycines*) (Atibalentja et al., 2000) with which it has the greatest similarity (96%).

The taxonomy of *Pasteuria* still remains unclear in part due to inconsistencies in the criteria used for differentiating the genus. There is variation in the shape of endospores and sporangia for isolates obtained from different nematode genera (Sayre and Wergin, 1977; Ciancio et al., 1994). Ultrastructure, morphology, life cycle, and host range are used as criteria for separating species of *Pasteuria*. These criteria are challenged by new isolates of *Pasteuria* spp. collected from plant and soil nematodes. Many *Pasteuria* isolates overlap in host specificity. As the size of endospores and sporangia is correlated with host nematode genera, these characters may not work for separating species (Ciancio et al., 1994). In addition, many *Pasteuria* isolates reported exhibit a high degree of morphological similarities and many others display cross-generic host ranges and varying biological and ecological characters (Chen and Dickson, 1998). The diameter of sporangia varies from 3 μm for an isolate from *Criconemella* sp. in

Florida (Han et al., 1999) to 8 μm for an isolate from *Axonchium valvulatum* in Sri Lanka (Ciancio et al., 1994).

Some recently discovered isolates of *Pasteuria* spp. parasitize multiple genera of nematodes with different biological and ecological characteristics. For example, isolates of *P. penetrans* reported from China (Pan et al., 1993), Puerto Rico (Vargas and Acosta, 1990), and the United States (Mankau, 1975; Oostendorp et al., 1990) parasitize both *Meloidogyne* spp. and *Pratylenchus* spp. In addition, one isolate reported from India parasitizes *Heterodera* spp. and *M. incognita* (Bhattacharya and Swarup, 1988), while another parasitizes *Globodera* spp., *Heterodera* spp., and *Rotylenchulus reniformis* (Sharma and Davies, 1996).

Various isolates of *Pasteuria* spp. parasitize only certain life stages of nematode host (Abrantes and Vovlas, 1988; Davies et al., 1990; Noel and Stanger, 1994). Davies et al. (1990) reported a *Pasteuria* sp. that could complete its life cycle in J2 of *Heterodera avenae*, but did not parasitize females or cysts. Abrantes and Vovlas (1988) reported an isolate of *Pasteuria* sp. that parasitizes juveniles and males of *Meloidogyne* sp. but only juveniles of *Heterodera fici*. Noel and Stanger (1994) stated that an Illinois isolate of *Pasteuria* sp. parasitizes J2 and males of *Heterodera glycines* but not females.

Sayre and Starr (1989) reported that *P. penetrans* could develop mature endospores only in females of *Meloidogyne* spp. However, one isolate of *Pasteuria* sp. has been reported developing mature endospores in juveniles, males, and females of *M. acronea* (Page and Bridge, 1985). Giblin-Davis (1990) indicated that an isolate of a *Pasteuria* sp. completes its life cycle in J2 of *Meloidogyne* spp. on turfgrass. However, they found that the same isolate, S-1 strain, did not attach to *H. schachtii*, *Longidorus*

africanus, *M. hapla*, *M. incognita*, *M. javanica*, *P. brachyurus*, *P. scribneri*, *P. neglectus* *P. penetrans*, *P. thornei*, *P. vulnus*, or *Xiphinema* spp.

Pasteuria spp. are reported to parasitize several genera of nematodes at the same location. Juveniles and adults of *Pratylenchus* sp. and *Tylenchorhynchus* sp., and second-stage juveniles of *Heterodera avenae* were infected with *Pasteuria* spp. in a nematode-suppressive soil in England (Davies et al., 1990). Whereas the endospores from the three nematode hosts were identical in size, it is uncertain if the endospores were from a single or multiple species of *Pasteuria.* The endospores obtained from J2 of *H. avenae* attached to J2 of *H. schachtii*, *H. glycines*, *Globodera rostochiensis*, *G. pallida*, and *M. javanica*, but none developed in females (Davies et al., 1990). In another example, a survey of sugarcane fields in South Africa revealed endospores of *Pasteuria* spp. attached to species of *Pratylenchus*, *Helicotylenchus*, *Scutellonema*, plus several other common soil nematodes (Spaull, 1981). Small endospores (2.9-4.4 × 1-2 µm) from *P. zeae*, *H. dihystera*, and J2 of *M. incognita* were assumed to be one strain of *P. penetrans*, whereas larger endospores (4.3-6.6 × 2.0 µm) from *Scutellonema* sp. and *Xiphinema* sp. were considered to be different strains.

Apparently ultrastructure, morphology, life cycle, and pathological characters are not sufficient to be used as criteria for differentiating the increasing number of *Pasteuria* isolates collected from soil and plant nematodes (Atibalentja et al., 2000). Additional studies are needed to resolve the current uncertainty with the taxonomy of *Pasteuria*. Information on *Pasteuria*'s genomic properties, such as size, base composition, and DNA sequence similarities revealed by hybridization, could help clarify the situation.

Cultivation of the bacteria in vitro could improve the understanding of its complex biology and taxonomy, but artificial cultivation has yet to be achieved.

Biology of *Pasteuria penetrans*

Life cycle

Meloidogyne spp. J2 becomes encumbered by endospores of *P. penetrans* as they move through soil. Attachment to the cuticle is the first step in parasitism, but for development to be complete there must be infection. Endospore germination is a critical step in the infective process. The penetration peg must pass through the cuticle and hypodermis thereby reaching into the nematode pseudocoelom. This process is temperature dependent and occurs 4 to 10 days after the nematode enters a plant root and begins to feed (Sayre and Wergin, 1977; Serracin et al., 1997). The germ peg is formed through the central opening in the basal attachment layer of the endospore. The penetration process appears to be enzymatic (Mankau, 1975; Mankau et al., 1976). Upon penetrating the pseudocoel of the nematode the germ peg develops into a "cauliflower-like" microcolony comprised of a dichotomously branched septate mycelium. Subsequently, daughter colonies form when the intercalary cells in the microcolony lyse (Sayre and Starr, 1989). As a result of an undetermined triggering mechanism, the colonies become fragmented where the terminal cells enlarge and undergo sporogenesis. Ultimately, quartets and doublets of developing sporangia fill the nematode pseudocoelom where they separate into single sporangia containing mature endospores. The mature endospores are released into soil when a plant root containing parasitized root-knot nematode females decomposes.

Sporogenesis

The sporogenesis sequence of *Pasteuria* includes the formation of a transverse septum within the endospore mother cell, condensation of a forespore, formation of cortex and coat, formation of parasporal fibers and exosporium, and finally, maturation and release of endospores. *Pasteuria* spp. have the typical sequence of a gram-positive, endospore-forming bacterium; however, mature endospores have a unique ultrastructure in each species (Chen et al., 1997; Sayre, 1993).

Systematics and phylogeny

In 1992, 13 genera of endospore-forming bacteria were known. Their systematics depends primarily on morphology, physiology, and diversity of genetic characters (Berkley and Ali, 1994). Currently, bacteria are differentiated based on the generally accepted rule that those with a DNA base composition differing by more than 10% GC (G+C) should not be considered as a member of the same genus. Strains differing by more than 5% GS values should not be considered as the same species (Bull et al., 1992). At present, the phenotypic characters are used more than molecular biology in classification and identification of prokaryotes. Recently, nucleic acid techniques have been used to determine bacterial genome properties, such as size, base composition, and the DNA sequence similarity revealed by hybridization. Nevertheless, limited information is available on the molecular biology (DNA base composition), and phylogeny of *Pasteuria* spp. because of the current difficulties in cultivating these bacteria in vitro (Chen and Dickson, 1998).

Some molecular evidence indicates that *P. penetrans* is a deeply rooted member of the *Clostridium-Bacillus* line of descendants, neither related to the actinomycetes nor closely related to the true endospore-formers (Berkeley and Ali, 1994). Anderson et al.

(1999) conducted a phylogenetic analysis using 16S rRNA derived from two isolates of *P. penetrans*. Their results suggest *P. penetrans* is closely related to *P. ramosa* with which it has 92% similarity. A lineage with *Alicyclobacillus* spp., *Sulfobacillus* spp., *Bacillus tusciae,* and *Bacillus schlegelii* is hypothesized (Anderson et al. 1999). Recently, phylogenetic analyses comparing the *Heterodera glycines*-infecting North American *Pasteuria* isolate with *P. ramosa* showed a 93% similarity of the two (Atibalentja et al., 2000). It is hypothesized that they form a distinct line of descendants within the *Alicyclobacillus* group of the Bacillaceae. Most recently, an analysis of 16S rRNA revealed that the *Pasteuria* strain that infects *B. longicaudatus* has a 98% similarity with *P. penetrans* (Bekal et al., 2001).

Host records

Pasteuria spp. have been observed in several groups of soil nematodes including tylenchids, dorylaims, rhabditids, mononchids, and aphelenchids (Sturhan, 1985). Chen and Dickson (1998) reported a more comprehensive review of the host records of *Pasteuria*-like organisms. They reported that *Pasteuria*-like organisms have been reported from 323 nematodes species belonging to 116 genera, including plant-parasitic, entomopathogenic, predatory, and free-living nematodes from more than 51 countries on five continents.

Host specificity

Opinions on the host specificity of *Pasteuria* spp. vary among scientists. The known host range of *P. penetrans* is limited to *Meloidogyne* spp. (Sayre and Starr, 1985; 1989). Stirling (1985) hypothesized that host specificity of *P. penetrans* might be a response to local nematode populations rather than to nematode species. Davies et al.

(1994) showed that *P. penetrans* could produce heterogeneous endospores, which show specificity to various nematode populations. Some of the records of host specificity were derived from observing attachment of endospores to the cuticle of nematodes rather than from observing actual infection and subsequent development inside the nematode (Oostendorp et al., 1990).

Endospore populations that possibly have a high degree of genetic diversity are commonly used to test host ranges, whereas work based on a single endospore isolate has not been reported. Consequently, considerable variation in host ranges has been reported among varies tests, duplicated tests, isolates of *Pasteuria* sp., and among generations of the same host nematode strain (Chen and Dickson, 1998). Host specificity of a particular isolate of *Pasteuria* sp. can be shifted from one nematode host to another by continually culturing the bacterium on the new nematode (Davies et al., 1988; Oostendorp et al., 1990). After such culturing treatments, endospores attached more readily to the nematode from which they originated. In contrast, tests with isolates from *M. javanica* and *M. incognita* showed that in some cases endospore attachment is unrelated to either species from which the endospores were obtained, or to the recipient nematode species (Stirling, 1985).

Status of the host specificity of *P. penetrans* remains unclear. Recent studies indicate that differences in the number and structure of proteins on the endospore surface and different characteristics (such as adhesion components) of the cuticle of nematode may be involved with attachment to a host (Brito et al., 2000; Davies et al., 1992; Davies, 1994; Mohan et al., 2001).

Cultivation

While commercial use of *P. penetrans* would most likely require an in vitro method of cultivation, currently available methods involve the multiplication of the parasite in its nematode host on greenhouse-grown plants (Stirling and Wachtel, 1980). It was thought that production could be improved by culturing the nematode and pathogen in excised or transformed roots cultures (Verdejo and Jaffee, 1988; Verdejo and Mankau, 1986), however, this method does not provide for release of endospores from females thereby limiting future generations.

Verdejo and Mankau (1986) reported a method for growing *P. penetrans* in *M. incognita* on excised tomato roots. An endospore-filled female nematode was squashed on a small block of agar. It was then placed close to the roots to release the endospores. Subsequently, a single *M. incognita* egg mass was added to the agar block. The endospores attached to the J2 before they penetrated the roots. After 58 days, infected female nematodes were found. Verdejo and Jaffee (1988) improved on this method by transforming tomato roots with *Agrobacterium rhizogenes*.

Several species of *Pasteuria* from *Pratylenchus brachyurus*, *Heterodera glycines*, and *M. incognita* were tested in a range of media for cultivation (Reise et al., 1988). Organic and mineral supplements were added to standard medium formulations, leading to increased production of mature endospores, sporangia, and vegetative cells. Several other researchers attempted various techniques to grow *Pasteuria* in vitro, but none has yet succeeded (Williams et al., 1989; Bishop and Ellar, 1991).

Ecology of *Pasteuria penetrans*

Temperature

The success of biocontrol of root-knot nematodes does not only depend on the biological knowledge of *Meloidogyne* spp. and *P. penetrans* relationships but it also depends on an understanding of conditions in the soil that favorable for the amplification of bacterium. The life cycle of *Pasteuria* species does not appear to be synchronized with its host nematode in that one can find all the developmental stages of the bacteria at one time within the nematode pseudocoelom (Chen et al., 1997). Attachment, infection, and pathogenesis of *Pasteuria* spp. is affected by temperature (Ahmed and Gowen, 1991; Hatz and Dickson, 1992; Serracin et al., 1997; Singh et al., 1990; Stirling, 1981; Stirling et al., 1990). Higher temperatures (30 and 35 ºC vs. 25 ºC or below) increased the rate of development (Hatz and Dickson, 1992; Serracin et al., 1997). The optimal temperature for development of an isolate of *P. penetrans* attained from *M. arenaria* race 1 was 35 ºC (Hatz and Dickson, 1992). Mature endospores of this isolate developed after 35, 40, 81, and 116 days at 35, 30, 25, and 20 °C, respectively (Hatz and Dickson, 1992), whereas another isolate attained from *M. arenaria* race 2 developed mature endospores after 28, 35, and 90 days at 35, 28, and 21 °C, respectively (Serracin et al., 1997). *P. penetrans* did not develop within females of *M. javanica* or *M. arenaria* at 10 °C (Hatz and Dickson, 1992). Temperature requirements do not appear to be consistent among different *P. penetrans* isolates. For example, an Indian isolate that infects *Heterodera* spp. and *M. incognita* completed its life cycle in *M. incognita* in 49 days at 10 to 17 °C (Bhattacharya and Swarup, 1988).

Temperature has been shown to affect endospore attachment to J2 (Ahmed et al., 1990; Singh and Dhawan, 1990; Stirling et al., 1990). The rate of endospore attachment at 27 °C is approximately double that at 18 °C (Stirling et al. 1990), with the maximum degree of attachment observed at 30 °C (Ahmed et al., 1990; Hatz and Dickson, 1992), and declining at higher temperatures (Hatz and Dickson, 1992). Another isolate of *Pasteuria* sp. that is parasitic on *H. cajani* showed higher numbers of endospores attached at 25 °C than at 15 or 35 °C (Singh and Dhawan, 1990). Freitas et al. (1997), showed that attachment of endospores to J2 was reduced after endospores were exposed to 40, 50, and 60 °C. Storage temperature and time periods also have been shown to affect attachment to J2. The endospore attachment to J2 of *Heterodera cajani* increased with increase storage temperature from 5 to 30 °C but declined after 45 °C (Gogoi et al., 2001).

Germination of endospores also is affected by temperature. Germ tubes formed and penetrated J2 of *M. arenaria* race 2, 9 to 10, 6, and 4 to 5 days after inoculation at 21, 28, and 35 °C, respectively (Serracin et al., 1997). How temperature affects germination is unclear; however, temperature may affect the development of host nematodes, subsequently triggering the germination of endospores.

Pathogenesis and endospores per root system were affected by temperature. At 30 °C, *P. penetrans* proliferated extensively through immature females, whereas at 20 °C females often developed ovaries containing eggs prior to infection, thus preventing development of *Pasteuria* (Stirling, 1981). At temperatures of 20, 25, 30, and 35 °C, the average number of endospores per root system was 12.5, 14.7, 115, and 113 million, respectively (Hatz and Dickson, 1992).

Development was affected by fluctuating temperature in different host plants (Giannakou et al., 1999). Greenhouse and growth room experiments showed that the host plant affected the development of *P. penetrans* indirectly through its effect on nematode development. The numbers of endospores produced per infected female of *M. javanica* were reduced with increasing numbers of females parasitizing okra and tomato roots. The life cycle of *P. penetrans* was completed faster at a constant temperature close to 30 °C than when the temperature fluctuated above or below 30 °C (Giannakou et al., 1999).

pH

Pasteuria penetrans was detected in acidic as well as in alkaline soils (Sturhan, 1985). Several studies have shown that effects of pH on endospore attachment rates vary. The optimum pH for attachment of endospores of *Pasteuria* spp. is between 7.0 and 8.5 (O'Brian, 1980). The highest number of attached endospores was found on *Heterodera cajani* at pH 8 in India (Kamra et al., 1998). Ahmed et al. (1990) found the highest attachment rate at pH 9, with decreasing rates at lower pH. Davies et al. (1988) observed a higher attachment rate at pH 7 than pH 4 or pH 9 in tap water, but the rate was lower at pH 7 than pH 4 or pH 9 in distilled water. Attachment of sonicated endospores was higher at pH 7 than pH 4 or pH 9 in distilled water and tap water, but for a given pH, attachment was higher with tap water (Davies et al., 1988). The endospore surface has a net negative charge, which was greatest at neutral pH and decreases at both a higher or lower pH (Afolabi et al., 1995). Consequently, Afolabi et al. (1995) concluded that previous reports of a negative electrostatic charge on the nematode cuticle must be in error (Himmelhoch et al., 1977) because attachment rates were highest when the negative charge on the endospore surface was highest.

Moisture

The effects of soil moisture on endospore attachment and development of *P. penetrans* are poorly understood. Since endospores of *P. penetrans* are not motile in soil, their attachment rate depends on the movement of J2 in soil. Endospore attachment is affected by soil moisture because soil moisture affects nematode movements (Van Gundy, 1985). However, there was no correlation between the number of endospores attached per J2 and the soil pore size or soil moisture levels in an experiment reported by Dutky and Sayre (1978). In contrast, an experiment to determine whether moistening air-dried soil containing *P. penetrans* for 3 days before adding J2 of *M. incognita* showed that endospore attachment increased by wetting the soil (Brown and Smart, 1984). Interestingly, soil moisture also affects the growth of *P. penetrans* within *Meloidogyne* spp. females. The development rate of *P. penetrans* in infected females decreased when soils were at or near saturation (Davies et al., 1991). A proposed explanation for this observation is that oxygen depletion in wet soil inhibits respiration, thereby inhibiting the development of the nematode and the bacterial parasite.

Soil type

Pasteuria penetrans was detected in a wide range of soil types varying from pure sand to organic soils (Sturhan, 1985). Laboratory experiments suggest that increasing sand content might improve endospore attachment (Singh and Dhawan, 1992). Sandy soil allows endospores to move downward with percolating water (Oostendorp et al., 1990; Chapter 3).

Survival

The long-term survival of endospores of *P. penetrans* in field soil has not been reported. The endospores resist various chemicals and environmental stresses in laboratory experiments. For example, endospores survived for more than 1 year at 10 to 36 °C (Mani, 1988). Endospores of *P. penetrans* show properties similar to those of other endospore-forming bacteria when subjected to various physical and chemical treatments. Examples include resistance to desiccation and sonication, and extrusion of endospore contents with prolonged exposure to 0.1% $KMnO_4$ in 0.3 N HNO_3 (Williams et al. 1989).

Endospores can survive under high temperature and desiccation (Williams et al., 1989). It is been reported that infectivity of *P. penetrans* endospores was reduced after heating endospores at 100 °C for 5 minutes, but attachment was not noticeably affected by heating at 100 °C for 15 minutes (Williams et al., 1989). Endospores also were resistant to desiccation and sonication. Dutky and Sayre (1978) observed that endospores attachment occurred at up to 80 °C, but infection did not occur at this temperature. Suspending endospores in water at temperatures higher than 30 °C for 5 hours daily over a 10-day period decreased attachment from 61 endospores/J2 at 30 °C to ≥ 8 endospores/J2 at 60 °C to 100 °C (Freitas et al., 1997).

Long-term survival of bacterial endospores is achieved by the following mechanisms: (1) a lack of high-energy compounds (ATP and NADH) in the endospore, (2) high concentrations of 3-phosphoglycerate (3PGA), dipicolinic acid (DPA), and divalent cations (Ca^{2+}, Mg^{2+}, and Mn^{2+}), (3) enzyme dormancy, (4) dehydration of the endospore protoplasm, and (5) presence of a thick cortex and coat (Setlow, 1994). The

combination of these factors is used to predict heat resistance. These include optimal growth temperature of the bacterium, water content of the endospore protoplasm, mineral content, and cortex size (Gerhardt and Marquis, 1989). The impermeability of the protoplasm membrane and endospore coat layers provides resistance to harmful chemicals (Setlow, 1994). Endospores of some bacteria can survive in soil up to 9,000 years (Nilsson and Renberg, 1990; Setlow, 1994). The known ultrastructure, morphology, sporogenesis, and chemical properties of endospores of *Pasteuria* spp. are comparable to other endospore-formers (Bird et al., 1990; Chen et al., 1997; Williams et al., 1989). However, endospores of *P. nishizawae* have a thinner cortex and coat layers compared with *P. penetrans*, thus they are expected to have less resistance to heat and certain chemicals (Nishizawa, 1989).

Pasteuria penetrans as a Biological Control Agent

Biological Control Potential

The parasitic characteristics of *P. penetrans*, which limits the infection and reproduction of root-knot nematodes, makes this bacterium a promising candidate as a biological control agent of the nematodes as opposed to other antagonists or methods (Chen and Dickson, 1998; Chen et al., 1996; Dickson, et al., 1994; Stirling 1984). The role of *P. penetrans* in reducing root-knot nematode populations has been observed on many crops, but mostly in greenhouse pots (Chen and Dickson, 1998). *Pasteuria penetrans* reduced *Meloidogyne* spp. on bean, chickpea, cucumber, eggplant, grape, hairy vetch, kiwi, mung, okra, peanut, pepper, rye, soybean, tobacco, tomato, and wheat (Chen and Dickson, 1998). *Pasteuria* spp. have been reported to suppress *Belonolaimus*

longicaudatus on bermudagrass turf (Giblin-Davis, 1990), and *H. elachista* on rice (Nishizawa, 1987).

There have been some unsuccessful cases involving isolates of *Pasteuria* spp. suppressing plant-parasitic nematodes. For example, Spaull (1984) indicated that in sugarcane fields in South Africa populations of *Meloidogyne* spp. were generally greater in fields infested with *P. penetrans* and that the level of parasitism was greater at higher densities of the nematodes. Also, Ciancio et al. (1992) showed that on turfgrass there was no significant relationship between the population density of *Helicotylenchus lobus* and the percentage of nematodes with endospores attached in soil naturally infested with *Pasteuria* sp. Both studies were surveys, so the findings were circumstantial. Fully designed experiments are needed to elucidate the role of *Pasteuria* spp. in suppressing nematodes in these particular cases.

Soil Suppressiveness

Suppressiveness of plant-parasitic nematodes in soils infested by biological agents is a relatively new idea that has only been studied during the past 2 decades. Suppressive soils are described as those in which disease development is suppressed even though the pathogen is introduced in the presence of a susceptible host (Huber and Schneider, 1982). Reported studies on suppressiveness of *Pasteuria* were based on either microplots or field research. In one microplot study, Chen et al. (1996) found that 10,000 endospores/g of soil was enough for suppression of *M. arenaria* race 1 on peanut. Also, a few nematode-suppressive soil sites infested with *P. penetrans* and root-knot nematodes have been documented (Dickson et al., 1991; Bird and Brisbane, 1988; Minton and Sayre, 1989; Stirling and White, 1982; Mankau 1980). Dickson et al. (1991) reported suppressiveness

of the peanut root-knot nematode in a peanut field near Williston, Florida. This particular field had been used for peanut nematode research for many years because of the heavy infestation of *M. arenaria* race 1. By the mid-1980s, peanut growing in the field began to show signs that the nematode population densities were declining. Slowly, over a period of 4 to 5 years peanut yield increased and root-knot nematode galling decreased significantly. They conducted numerous tests to determine whether biological control agents in the soil caused the suppressiveness. These tests revealed that *P. penetrans* was a major factor in suppressing the population densities of *M. arenaria*.

Efficacy and Factors Influencing the Population Dynamics of *Pasteuria penetrans*

Greenhouse and microplots tests consistently have shown that *P. penetrans* reduces root-knot nematode galling and egg production (Daudi et al., 1990; Vargas et al., 1992). Infection by *P. penetrans* reduces the number of J2 penetrating roots (Davies et al., 1988) because their movement is significantly reduced when they are encumbered with an average of seven or more individual endospore (Davies et al., 1991). Also, infection of females by *P. penetrans* endospores reduces the numbers in roots (Davies et al., 1991), their fecundity (Bird and Brisbane, 1988), which ultimately lead to fewer J2 in soil (Davies et al., 1988), and fewer eggs masses on roots (Ahmed and Gowen, 1991; Weibelzahl-Fulton et al., 1996).

In order to capitalize on soils that are naturally suppressive because they are infested with large densities of *P. penetrans* we need to understand the population ecology of the bacterium. A few studies have shown that some nematode management practices, such as solarization, soil amendments, or soil fumigation may compromise or deteriorate the efficacy of *P. penetrans* (Freitas et al., 2000a; 2000b). In particular, soil

fumigants that contain chloropicrin have been shown to be bactericide (Freitas et al., 1999). Although attachment is not reduced, development inside the nematode is completely shut down (Freitas et al., 1999).

History of a *P. penetrans* Suppressive Field

A field site located at the Woodrow Fugate farm near Williston, Florida was identified as a *P. penetrans* root-knot nematode suppressive site in mid-1980s (Dickson et al., 1991). The site had been used for peanut nematode research beginning in 1970. At that time and over the next 10 to 15 years the site was conducive for root-knot of peanut. In the beginning there was a heavy infestation of *M. arenaria* race 1, and peanut yields in untreated plots were generally always low compared with plots treated with fumigant nematicides. The site began to show signs that the severity of nematode disease was declining some time around mid-1980s. Gradually, over a period of 4 to 5 years, peanut yield increased and root-knot nematode galling on pegs and pods decreased greatly (Dickson et al., 1991; Dickson et al., 1994). *P. penetrans* was reported to have been one of the main factors causing the suppressiveness of the soil (Dickson et al., 1994). Examination of the second-stage juveniles (J2) extracted from soil taken across the field showed that 32% of them were infected with one or more endospores of *P. penetrans.* Without any soil treatments, peanut yields increased from about 2000 kg/ha in early 1980s to over 5,605 kg/ha in 1990.

Objectives

Biological control is an attractive alternative to chemical control. It offers exciting possibilities for the future management of nematodes. Recently, *P. penetrans*, an endospore-forming bacterial obligate parasite of nematodes, has been recognized as a

potential biological control agent for *M. arenaria* race 1. *Pasteuria penetrans* is known to cause soils to become suppressive to this nematode (Dickson et al., 1994).

The long-term goal was to study the persistence and suppressiveness of *P. penetrans* (isolate P-20) to *M. arenaria* race 1, but during the course of the study *M. javanica* was discovered infecting peanut at the experimental site. Hereafter they are referred to as *Meloidogyne* spp. because of the mixture of the two species.

The objectives of the research reported herein were (i) to determine the long-term effects (9 years) of two nonhost crops and weed fallow on *M. arenaria* race 1 and *P. penetrans* population densities, (ii) to determine the suppressiveness of the soil to *M. arenaria* race 1 and the length of time that suppressiveness is maintained, (iii) to determine downward percolation of *P. penetrans* endospores vertically in soil in the field and in the laboratory, (iv) to determine the percentage of *Meloidogyne javanica* in the peanut field (based on esterase phenotypes), and (v) to confirm that *M. javanica* infects peanut at this site.

CHAPTER 2
EFFECT OF TWO CROPS AND WEED FALLOW ON *PASTEURIA PENETRANS* IN FIELD SOIL

Introduction

The peanut root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood race 1, is described as one of the most important pathogens of commercially grown peanut (*Arachis hypogaea* L.) in the United States, especially in the southeastern United States, and throughout the world (Minton, 1984). Currently, nematicides and crop rotation are currently the only control tactics for root-knot nematodes in peanut (Kinloch, 2001), although plant resistance is a promising new area of research (Nelson et al., 1989; Holbrook and Noe, 1990; Shew et al.1993; Simpson and Starr, 2001). Biological control of nematodes with microbial agents also is an attractive alternative to chemical control, and this area of research is receiving increased interest among nematologists. It offers strong potential for future management of nematodes (Dickson, 1998).

A peanut field located on the Woodrow Fugate farm near Williston, Levy County, FL, has been used for peanut nematode research for many years beginning in 1970. At that time root-knot was a devastating disease on the site (Dickson, pers. comm.). Around the mid-1980s the site began to show signs that the nematode disease problem was declining. Gradually, over a period of several years peanut yield increased and root-knot nematode galling on roots, pegs and pods decreased greatly (Dickson et al., 1991; Dickson et al., 1994). Examination of second-stage juveniles, (J2) extracted from soil samples taken across the field showed that 32% of them had high numbers of endospores

of *Pasteuria penetrans* attached. In 1990, the entire 2-hectare site was planted with peanut and the yield averaged 5,605 kg/ha, which was well above the state average in Levy County for that year (1990). The conclusion was that the site had become highly suppressive to the peanut root-knot nematode and the most likely reason was that *P. penetrans* had built to a highly suppressive density.

There are few or no data on the long-term persistence of *P. penetrans* in suppressive sites and whether or not suppressiveness can be maintained for a long period of time. Also, no data were found in the literature on the effect of growing various crops, some nonhosts for the nematode, on the bacterium, or the suppressiveness of soils. These become important considerations if we are to understand soil suppressiveness to plant-pathogenic nematodes. We need to know how long suppressiveness is maintained, and the effects of different plants are growing in suppressive soils? In an attempt to answer these concerns it was decided that a randomized complete block design be established at the suppressive site of the Fugate farm. Treatments imposed were two nonhost crops for *M. arenaria* race 1, bahiagrass (*Paspalum notatum* Flugge cv. Pensacola var. Tifton 9) and rhizomal peanut (*Arachis glabrata* Benth. cv. Florigraze), and weed fallow beginning in the summer of 1991. The objectives were to determine the long-term effects (9 years) of two established crops, bahiagrass and rhizomal peanut, and weed fallow on persistence of *M. arenaria* and *P. penetrans*.

Materials and Methods

A RCBD with 10 replications was established in 1991. The three treatments were bahiagrass, rhizomal peanut, and weed fallow. Two root-knot nematodes non-host weeds observed in weed fallow plots were hairy indigo (*Indigofera hirsuta*) and alyce clover

(*Alysicarpus vaginalis* (L). All plots were planted with rye (*Secale cereale* L. cv. Wrens Abruzzi) during the autumn-winter season as a cover crop. Each plot was 38 m long, and 10.6 m wide with a 2.4 m wide non-tilled border separating each plot. The soil was classified as Arredondo fine sand (92.5% sand, 4% silt, 3.5% clay, and <1% organic matter; pH 7.2). From 1991 to 1999, the site received minimum inputs. No fertilizer, irrigation, or pest-pathogen management tactics were applied; however, once or twice each year weeds were mowed in the weed fallow plots and in all borders and alleyways. Bahiagrass established well during the first two growing seasons, but rhizomal peanut required about 3 years to give complete ground cover.

Development of *Meloidogyne arenaria* race 1 and *Pasteuria penetrans*. Experiments on the population densities, persistence, and suppressiveness of a field soil infested with *M. arenaria* race 1 and *P. penetrans* were begun in 1999, and continued through 2002. Peanut was planted in the bahiagrass and weed fallow plots for four consecutive years (1999 to 2002), and in the rhizomal peanut plots three concecutive years (2000 to 2002). In 1999, the bahiagrass and weed fallow plots three were prepared for planting peanut by mowing, plowing, and disking. The rhizomal peanut plots could not be plowed or disked because of a deep and thick thatch layer. Therefore, glyphosate herbicide was sprayed over the rhizomal peanut plots in the summer of 1991 to kill the plants and the plots were prepared for planting to peanut in 2000. The Florunner cultivar was planted in 1999, the Southern Runner cultivar in 2000, and the Georgia Green cultivar in 2001 and 2002. Each plot consisted of 10 rows planted on 90-cm row spacing. Production practices common for growing peanut in the area were applied (Whitty, 2002).

Twelve cores (2.5 cm-diam., 20 cm-deep) of soil were taken from each plot each growing season with a cone-shaped sampling tube 1 to 3 days before planting (Pi), near mid-season [55-65 days after planting (Pm)], and at final harvest (Pf). Soil cores from each plot were combined, mixed thoroughly and nematodes were extracted from a 100 cm^3 subsample by a centrifugal-flotation method (Jenkins, 1964). All plant-parasitic nematodes were counted. The first 20 J2 observed from Pi (spring) and Pf (autumn) populations were used to determine the number of *P. penetrans* endospores attached. Twenty-six plants were chosen randomly from each plot at harvest every season to determine root-knot nematode galling based on an scale of 0 to 100 (0 = no galls on roots and 100 = 100% of the root system galled) (Barker et al., 1986). Plant growth ratings were determined at or near harvest based on a scale of 1 to 10 (1 = poor growth and 10 = good growth). At the end of each season, except 2002, peanut plants were dug, left on the soil surface for 1 or 2 days to dry, and harvested with a peanut combine. Yield data were recorded from the middle six rows (total of 10 rows in a plot) of each plot. Although all peanut plants were dug in 2002, yields were not determined because plants in all plots were severely stunted from drought during the first 2 months of the growing season.

Soil bioassay for *pasteuria penetran.* A soil bioassay was conducted preplant and at harvest to determine the presence of *P. penetrans* in the soil. A portion of soil from the soil samples (above paragraph) taken from each plot was air-dried and 40 g from each sample was placed in a 50-ml sterile polyethylene centrifuge tube. Soil water content was adjusted to 100% (saturated) capacity to increase the rate of endospore attachment to J2 (Brown and Smart, 1984). Then 500, 1-to 3-day-old J2 of *M. arenaria* race 1 were

added and the tubes left uncovered at room temperature. Three days later the J2 were extracted by a centrifugal-flotation method (Jenkins, 1964). The following scale was used to estimate the number of endospores attached per J2: 0 = none, 1 = 1-2, 2 = 3-5, 3 = 6-15, 4 = 16-100, 5 = >100. Observations were made with an inverted microscope and data were recorded from the first 20 J2 observed per sample.

Origin of the root-knot nematode isolate. The isolate of *Meloidogyne arenaria* race 1 used in the bioassay originated from peanut grown at the former University of Florida Green Acres Agronomy Farm, Alachua County, FL. The nematode was cultured on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers). Eggs of *M. arenaria* race 1 were collected from galled roots by treating them with 0.5% sodium hypochlorite (Hussey and Barker, 1973). Eggs and J2 were caught on a sieve with 25 µm-pore openings. The eggs were hatched on a modified Baermann funnel (Rodríguez-Kábana and Pope, 1981) and used as 1-to 3-day-old J2 for the bioassays.

Number of females of *Meloidogyne arenaria* race 1 infected with *Pasteuria penetrans*. Galled roots from 26 peanut plants were collected from each plot at harvest and kept in a paper bag until they were processed. After 5 to 6 weeks, about 10 g of dry roots from each sample were incubated for 2 days in an enzymatic maceration solution. The solution consisted of 50 ml of 0.5 M Na acetate (pH 5.0), 50 ml of Pomaliq 2 F (Gist-Brocades, Charlotte, NC), 500 µl of 1.0 M $CaCl_2$, and 400 ml deionized water (Charnecki, 1997). The samples were washed vigorously over stacked-sieves with 30-µm-pore openings (top) and 200-µm-pore openings (bottom). Forty females were picked at random from the females collected on the bottom sieve. These females were crushed

on a glass slide, covered with a cover slip, and checked for the presence of *P. penetrans* endospores.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC) and mean treatment differences were separated and compared using Duncan's multiple-range test. Linear regression lines were run with Microsoft Excel (Microsoft Corporation, Redmond, WA).

Results

Treatments affected the initial and final population densities of *Meloidogyne* spp. (*M. arenaria* and *Meloidogyne javanica*) ($P \leq 0.05$) (Table 2-1). Initial densities were greater ($P \leq 0.05$) in the weed fallow plots than in the bahiagrass and rhizomal peanut plots. Also, population densities (Pi, Pm, and Pf) were different among years, and years × treatments interaction ($P \leq 0.05$) (Table 2-1). In 2000, the Pi density remained low in the rhizomal peanut plots, but increased in the bahiagrass and weed fallow plots ($P \leq 0.05$). In general, the densities of *Meloidogyne* spp. remained relatively low throughout the course of the experiment. *Meloidogyne* spp. reached the highest densities numerically at the Pf of the 3rd year (2001) in all three treatments. There was a gradual increase in root-knot nematode densities in the rhizomal peanut plots with the largest increases observed in the 3rd and 4th seasons.

Treatments, years, and treatments and years interaction affected the percentage of J2 with endospore attached and the average number of endospores per J2 in both spring and autumn seasons ($P \leq 0.05$) (Table 2-2). The percentage of J2 with endospores attached and the average number of endospores per J2 were nearly undetectable in all

Table 2-1. The initial (Pi), mid-season (Pm), and final (Pf) population densities of *Meloidogyne* spp[a] based on 100 cm^3 of soil taken from peanut grown over four consecutive summer seasons (1999-2002) following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

Treatments[b]	Initial population (Pi)	Mid-season population (Pm)	Final population (Pf)
		1999	
Bahiagrass	1 b	31 b	183 a
Rhizomal peanut	4 b	40 b	2 b
Weed fallow	17 a	147 a	214 a
		2000	
Bahiagrass	13 ab	172 a	161 a
Rhizomal peanut	1 b	49 b	41 b
Weed fallow	25 a	165 a	106 a
		2001	
Bahiagrass	15 a	137 a	459 a

Table 2-1. Cont'd.

Rhizomal peanut	11 a	123 a	200 b
Weed fallow	13 a	98 a	309 ab
		2002	
Bahiagrass	44 a	109 ab	130 a
Rhizomal peanut	34 a	193 a	155 a
Weed fallow	35 a	51 b	177 a
		ANOVA	
Treatments (T)	*	ns	*
Years (Y)	*	*	*
T×Y	*	*	*

[a]*Meloidogyne arenaria* race 1 is the dominant species infecting peanut at this site; however, *M. javanica* also was discovered infecting peanut in this field in the autumn of 2001 (see Chapter 5).

[b]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer.

Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

Table 2-1. Cont'd.

Data are an average of 10 replications. Means within each column in the same year with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

Table 2-2. The percentage of second-stage juveniles (J2) of *Meloidogyne* spp.[a] with endospores of *Pasteuria penetrans* attached and the average number of endospores attached per J2 in 100 cm^3 of soil collected from each peanut plot before planting and at harvest for four growing seasons (1999-2002) following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

Treatments[b]	Percentage of J2 with endospores attached	Average number of Endospores/J2	Percentage of J2 with endospores attached	Average number of Endospores/J2
	Spring 1999		Autumn 1999	
Bahiagrass	0 a	0.0 a	0 a	0.0 a
Rhizomal peanut	0 a	0.0 a	0 a	0.0 a
Weed fallow	2 a	0.1 a	0 a	0.0 a
	Spring 2000		Autumn 2000	
Bahiagrass	1 a	0.1 a	10 b	1.1 a
Rhizomal peanut	0 a	0.0 a	3 b	0.3 a
Weed fallow	7 a	0.2 a	32 a	0.1 a

Table 2-2. Cont'd.

	Spring 2001		Autumn 2001	
Bahiagrass	3 a	0.1 a	7 b	0.2 b
Rhizomal peanut	2 a	0.1 a	2 b	0.3 b
Weed fallow	7 a	0.1 a	75 a	5.8 a
	Spring 2002		Autumn 2002	
Bahiagrass	12 ab	0.3 a	29 a	2.1 ab
Rhizomal peanut	4 b	0.1 a	21 a	0.6 b
Weed fallow	19 a	0.3 a	39 a	4.2 a
	ANOVA			
Treatments (T)	*	*	*	*
Years (Y)	*	*	*	*
T×Y	*	*	*	*

[a]*Meloidogyne arenaria* race 1 is the dominant species infecting peanut at this site; however, *M. javanica* also was discovered infecting peanut in this field in the autumn of 2001 (see Chapter 5).

Table 2-2. Cont'd.

[b]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer. Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

Data are an average of 10 replications with 20 J2 per replication. Means with the same letter within each column in the same year and the same season are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

plots in 1999 and the spring of 2000; however, they increased in all plots in the autumn of 2001 with the largest increase occurring in the weed fallow plots ($P \leq 0.05$) (Table 2-2). In the spring and autumn of 2002, the percentage of J2 with endospores attached were lower in the rhizomal peanut plots than in the weed fallow plots, and also in the autumn there were fewer endospores attached per J2 in the rhizomal peanut plots compared with the weed fallow plots ($P \leq 0.05$) (Table 2-2). There were no differences among the other treatments (Table 2-2).

Soil bioassays conducted over the 4 years showed a positive linear relationship with *Pasteuria* endospores and sampling dates (Fig. 2-1). There was an increase in the number of endospores per J2 in all plots over the 4-year period (weed fallow: Y = 0.4461x - 0.6698, $R^2 = 0.8755$, $P \leq 0.05$, bahiagrass: Y = 0.3068x - 0.7568, $R^2 = 0.8096$, $P \leq 0.05$, and rhizomal peanut: Y = 0.1452x - 0.3978, $R^2 = 0.6179$, $P \leq 0.05$). The greatest increase in densities of endospores occurred in the weed fallow plots, and the lowest densities occurred in the rhizomal peanut plots ($P \leq 0.05$) (Fig. 2-1).

Treatments affected the number of endospores per J2 in all the six-endospore categories except for category 1 (1-2 endospore/J2) ($P \leq 0.05$) (Table 2-3). Also, years affected the number of endospores per J2 in the all endospore categories except for category 2 (3-5 endospore/J2) over the 4-year ($P \leq 0.05$) (Table 2-3). Treatments × years interaction also affected the number of endospores per J2 differently in all categories ($P \leq 0.05$) (Table 2-3). In 1999, weed fallow plots supported a greater percentage of J2 with endospores attached and average number of endospores per J2 with the greatest numbers observed in the first four categories (0, 1, 2, 3, -4) ($P \leq 0.05$) (Table 2-3). In 2000, there were differences in percentage of J2 with endospores attached among all three treatments,

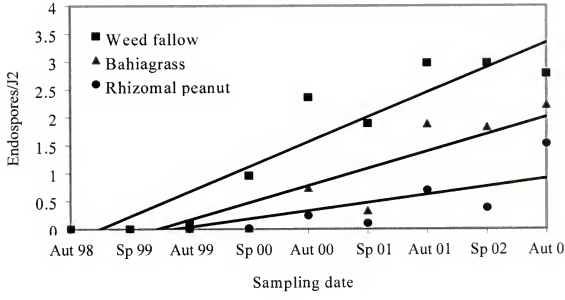

Fig. 2-1. Bioassay of the number of endospores of *Pasteuria penetrans* attached to second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 in soil collected at planting and at harvest of peanut grown each year for 4 consecutive years in weed fallow, bahiagrass, and 3 years in rhizomal peanut plots. Data were transformed with $\log_{10}(x+1)$ before analysis. A linear model described a positive response between log-transformed *P. penetrans* endospore numbers and sampling dates for each of three treatments. Weed fallow: $Y = 0.4461x - 0.6698$, $R^2 = 0.8755$, $P \leq 0.05$, bahiagrass: $Y = 0.3068x - 0.7568$, $R^2 = 0.8096$, $P \leq 0.05$, and rhizoma peanut: $Y = 0.1452x - 0.3978$, $R^2 = 0.6179$, $P \leq 0.05$ (Aut = Autumn; Sp = spring).

Table 2-3. Soil collected at peanut harvest (1999-2002) was used to conduct a bioassay[a] of the percentage of second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 with endospores of *Pasteuria penetrans* attached and the average number of endospores attached per J2 following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

Treatments[b]	Percentage of J2 with endospores attached[c]	Endospores/J2[c]	Category (endospore/J2[d])					
			0 (None)	1 (1-2)	2 (3-5)	3 (6-15)	4 (16-100)	5 (>100)
		1999						
Bahiagrass	2 b	0.2 b	98 a	2 b	0 b	0 b	0 a	0 a
Rhizomal peanut	2 b	0.2 b	98 a	2 b	0 b	0 b	0 a	0 a
Weed fallow	63 a	0.9 a	27 b	38 a	22 a	13 a	0 a	0 a
		2000						
Bahiagrass	50 b	0.8 b	50 b	30 a	13 b	6 c	1 b	0 a
Rhizomal peanut	22 c	0.3 c	80 a	19 b	1 c	0 b	0 b	0 a
Weed fallow	87 a	2.4 a	23 c	25 ab	20 a	17 a	15 a	0 a
		2001						

Table 2-3. Cont'd.

Bahiagrass	63 ab	1.9 b	39 ab	10 b	10 a	20 a	21 b	0 a
Rhizomal peanut	47 b	0.7 c	53 a	24 a	12 a	6 b	5 c	0 a
Weed fallow	79 a	3.0 a	27 b	3 b	11 a	13 ab	45 a	1 a
			2002					
Bahiagrass	63 ab	2.2 a	36 ab	5 a	11 a	16 a	31 ab	1 a
Rhizomal peanut	53 b	1.5 b	46 a	12 a	12 a	16 a	14 b	0 a
Weed fallow	77 a	2.8 a	21 b	8 a	11 a	13 a	45 a	2 a
			ANOVA					
Treatments (T)	ns	*	*	ns	*	*	*	*
Years (Y)	*	*	*	*	ns	*	*	*
T×Y	*	*	*	*	*	*	*	*

Table 2-3. Cont'd.

[a]Forty grams of air-dried soil was placed in a 50 ml sterile polyethylene centrifuge tube. Five-hundreds, 1 to 3 day-old *Meloidogyne arenaria* race 1 J2 were added. Three days later, J2 were extracted by a centrifugal-flotation method and the number of endospores attached per J2 was counted based on the first 20 J2 observed per sample.

[b]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer. Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

[c]Data are means of four replications with 20 J2 per replication and were transformed with $\log_{10}(x+1)$ before analysis. Means within a column in the same year followed by the same letter are not different according to Duncan's multiple-range test ($P \leq 0.05$).

[d]Percentage of J2 with endospore attached based on 20 nematodes per plot divided by six categories (category 0 = none endospores attached per J2, category 1 = 1 to 2 endospores attached per J2,……..category 5 = >100 endospores attached per J2). Data were transformed with arcsin ($\sqrt{x}$) before analysis, but untransformed arithmetic means are presented.

ns = nonsignificant at $P \leq 0.05$.

with the greatest numbers occurring in weed fallow plots ($P \leq 0.05$) (Table 2-3). The greatest percentages of J2 with endospores attached were observed in weed fallow plots in 2000 (87%). The largest average number of endospores per J2 was observed in weed fallow plots in 2001 (3 endospores/J2). The 3rd and 4th years supported the greatest percentages of J2 with endospores attached in all treatments, and, the greatest numbers of endospores attached per J2.

There was an effect of treatments, years, and year × treatments interaction on galling percentages ($P \leq 0.05$) (Table 2-4). There was consistently high gall rating on peanut for each treatment over the 4 years; but in 2002 the least amount of galling occurred in the weed fallow plots ($P \leq 0.05$) (Table 2-4). Galling was high on peanut grown in bahiagrass plots even the first year and remained fairly consistent over the 4 years, whereas in the rhizomal peanut plots galling was around 50% in years 2 and 3 but increased to over 90% in the 4th year (Table 2-4).

There was no difference among treatment effects on the plant growth ratings within a year, but years, and year × treatments interaction affected plant growth rating ($P \leq 0.05$) (Table 2-4). The growth rating of peanut was low in all plots throughout the study except for the rhizomal peanut plots in 2000 and 2001 ($P \leq 0.05$) (Table 2-4). There were differences in growth ratings among the treatments in each of 4years except for 1999 ($P \leq 0.05$) (Table 2-4).

There was a difference among treatment effects on yield, but this difference varied from year to year ($P \leq 0.05$) (Table 2-4). There also was a difference among years, and year × treatments interaction on yield ($P \leq 0.05$) (Table 2-4). In 1999, peanut yield was higher in bahiagrass plots than in weed fallow plots ($P \leq 0.05$), and this trend

Table 2-4. Comparison of overall galling percentages, growth rating, and yield of peanut grown each year from 1999 to 2002 following 9 years of bahiagrass, rhizomal peanut, and weed fallow.

	1999			2000			2001			2002		
Treatments[a]	% Galling[b]	Growth rating[c]	Yield kg/plot	% Galling	Growth rating	Yield (kg/plot)	% Galling	Growth rating	Yield (kg/plot)	% Galling	Growth rating	Yield (kg/plot)
Bahiagrass	87 a	3.8 a	19 a	96 a	3.6 b	5 b	80 a	3.2 c	13 c	72 b	3.2 ab	ND
Rhizomal peanut	nd	nd	nd	55 b	8.0 a	12 a	53 b	6.7 a	22 a	94 a	2.3 b	ND
Weed fallow	85 a	3.5 a	12 b	99 a	3.3 b	3 c	71 a	4.9 b	16 b	52 c	3.8 a	ND
					ANOVA							
Treatments (T)	*	ns	ns									
Years (Y)	*	*	*									
T×Y	*	*	*									

[a]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer.

Table 2-4. Cont'd.

Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

[b]Root-galling percentages were determined on 26 plants per plot at harvest based on a scale of 0 to 100 (0 = no galls; 1 = 1%....100 = 100% of root system galled).

[c]Peanut growth was rated on a scale of 1 to 10 (1 = stunted, chlorotic, or dead plants, 10 = full, green, lush growth).

ND = No data because of drought conditions in 2002.

nd = No data because of a profound and heavy thatch layer prevented plowing under the rhizomal peanut plots. Glyphosate herbicide was sprayed over the rhizomal peanut and the plots were prepared for planting peanut in 2000.

Data are an average of 10 replications. Means within each column with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

Table 2-5. Number of *Pasteuria penetrans* endospore-filled females[a] of *Meloidogyne arenaria* race 1 collected from peanut roots grown during the 2000-2002 seasons following 9 years of bahiagrass, rhizomal peanut, and weed fallow.

	Percentage of *Meloidogyne arenaria* race 1 endospore-filled females		
Treatments[b]	2000	2001	2002
Bahiagrass	6.0 b	11.8 b	41.2 b
Rhizomal peanut	0.3 b	1.3 c	16.0 c
Weed fallow	42.5 a	53.3 a	56.0 a
		ANOVA	
Treatments (T)	*	*	*
Years (Y)	*	*	*
T×Y	*	*	*

[a]The first 40 females were observed for the presence of endospores inside the body. No data were reported for 1999 because no females were found with endospores.

[b]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer.

Table 2-5. Cont'd.

Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

Data are an average of 10 replications. Means within each column with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

continued in 2000, but in 2001 yield was less in bahiagrass than in weed fallow plots ($P \leq 0.05$) (Table 2-4). The highest increase among treatments in peanut yield was observed in rhizomal peanut plots in 2001, the last year of recorded yield data ($P \leq 0.05$) (Table 2-4).

Treatments, years, and treatment × years interaction affected the percentage of *P. penetrans* infected females for the last 3 years of the 4-year study ($P \leq 0.05$) (Table 2-5). In 1999, no endospore-infected females were recorded, but for the last three years, the percentage of endospore-infected females consistently increased in numbers in all plots over the course of the study (Table 2-5). In 2000, weed fallow plots supported a greater numbers of endospore-infected females than bahiagrass or rhizomal peanut plots and this trend held for years 2001 and 2002 ($P \leq 0.05$) (Table 2-5). Rhizomal peanut plots maintained the lowest number of endospore-infected females in both 2001 and 2002 ($P \leq 0.05$) (Table 2-5). Bahiagrass plots supported the greatest increase in the number of endospore-infected females increasing from 6% in 2000 to 41% in 2002 ($P \leq 0.05$) (Table 2-5).

Discussion

Peanut grown in bahiagrass, rhizomal peanut, and weed fallow plots affected the number of J2 of *Meloidogyne arenaria* race 1 and endospore densities of *Pasteuria penetrans* in the 4-year study. At the beginning of the experiment, *P. penetrans* was detected in very low numbers in the soil, but the number of root-knot nematodes also was low. Densities of endospores are likely dependent on root-knot nematode densities (Dickson et al., 1991; 1994; Sayre and Starr 1993). Our bioassays indicated that the presence of *P. penetrans* in the soil could be detected with greater precision using the

bioassay than by detecting endospore-encumbered J2 extracted from soil. Air drying of soil, the first step of the bioassay procedure, could inhibit or terminate other microbial activities that could hinder endospore attachment. Or it may be a matter of maturation of endospores. Bringing the soil moisture content up to 100% in a bioassay positively influences attachment (Brown and Smart, 1984). It is known that endospores that have shed the exosporium coat readily attach to the nematode host (Davies et al., 1988; Stirling, 1985).

Over the course of the experiment, weed fallow plots supported the greatest endospore densities, whereas rhizomal peanut resulted in the lowest densities, with bahiagrass being intermediate. Since neither bahiagrass nor rhizomal peanut are hosts for *M. arenaria*, they are suggested as rotation crops for management of that nematode in peanut (Norden et al., 1977; Prine, 1978; Sumner et al., 1999). In weed fallow plots, two weeds, hairy indigo (*Indigofera hirsuta*) and alyce clover (*Alysicarpus vaginalis* (L), were observed supporting *M. arenaria* race 1. The hairy indigo population was relatively dense in the weed fallow plots, a few plants occurred in the bahiagrass plots, but they were rare in the rhizomal peanut plots. While hairy indigo has been reported to suppress *M. javanica* and *M. incognita* (McSorley et al., 1994; Rodríguez-Kábana et al., 1988), this weed is susceptible to *M. arenaria* race 1. Alyce clover also was heavily galled in weed fallow plots and is known to support development of the commonly occurring root-knot nematodes. Development of *M. arenaria* race 1 on these two weeds would most likely sustain *P. penetrans*. Thus, variability among endospores densities became more apparent among the treatments as the study progressed over 4 years despite the fact that the endospore densities were almost undetectable at the beginning, in 1999. With the

current methods available for detecting *P. penetrans* in soil, only a low precision of detection is thought to be possible.

The population densities of *P. penetrans* increased over time in all plots from almost undetectable levels to substantially high levels. Nonetheless, there was no strong evidence that endospore densities reached suppressive levels since peanut roots, pods, and pegs were significantly galled, and yields were greatly suppressed. Unfortunately, in the final year of the experiment an extended drought caused extreme stress on plants to the point where harvesting was not possible.

Additionally, the examination of peanut roots revealed infection by *Rhizoctonia solani* Kuhn (*Rhizoctonia* root and stem rot disease). This fungal agent occurs worldwide and causes stand loss when the soil-fungi population is substantially high (Porter et al., 1984). However, the density of this disease on peanut was not determined. But it was not surprising that the disease became significant because peanut was grown in the field mostly in a monoculture from 1969 through 1990, and then during the past 4 years. Still, over the course of the study peanut yield were better in the bahiagrass and rhizomal peanut than in the weed fallow plots.

Two successful examples of the biological control potential of *P. penetrans* for management of peanut root-knot nematode on peanut were reported recently when the bacterial agent was introduced to field microplots (Chen et al., 1996; Oostendorp, 1990, 1991). These two studies demonstrated that *P. penetrans* suppressed the peanut root-knot nematode in a period of 2 or 3-years, and resulted in the soil having very low numbers of J2, very low root, pod and peg galling, and significant increases in peanut yield. In

another example, *P. penetrans* suppressed the peanut root-knot nematode beginning from the third season of a 7-year study (Weibelzahl-Fulton, 1998).

Meloidogyne javanica was discovered infecting peanut in this field and that was totally unexpected (Cetintas and Dickson, 2003a; 2003b; Lima et al., 2002; Chapter 5). Its detection in the early autumn of 2001, following peanut harvest, means that a portion of peanut yield loss each year could be attributed to this nematode (Lima et al., 2002; Chapter 5). The presence of *M. javanica* could mean that soil suppressiveness by *P. penetrans* as previously reported (Dickson et al., 1991) could not again be reached. It is not known whether *P. penetrans* P-20 isolate attaches to and develops on *M. javanica* race 3, however it is not thought to be a host (Oostendorp et al., 1990)

The long-term persistence of *P. penetrans* and suppressiveness in soil is an unknown. This was the first attempt to study the effects on the development of *P. penetrans* in natural field soil, i.e. without the introduction of either root-knot nematode or *P. penetrans*. In the 4-year study, there were relatively low root-knot nematode population densities, which may be attributed to infection and suppression by *P. penetrans*. These results warrant additional long-term experiments with extended time to aid in the understanding of the dynamics of *P. penetrans* in field soil, and to determine whether nematode population densities can be maintained at acceptable low levels.

CHAPTER 3
DISTRIBUTION AND DOWNWARD PERCOLATION IN FIELD SOIL OF *PASTEURIA PENETRANS* ENDOSPORES

Introduction

Pasteuria penetrans (Thorne) Sayre & Starr, an obligate parasite of root-knot nematodes, has shown potential as a biological control agent (Chen and Dickson, 1998). It is at least one of the agents that causes soils to become suppressive to root-knot nematodes (Dickson et al., 1991; Dickson et al., 1994).

The distribution of endospores in soil may play an important role in the efficacy and success of the organism as a biological control agent. Although endospores of *P. penetrans* can move downward in soil when water is applied (Ostendorp et al., 1990; Dickson et al., 1994), little information is available on the organism's long-term persistence, distribution, and movement in soil. Percolation by rainwater could affect distribution of endospores and even flush endospores from the upper 30-cm of soil where they are likely to be most effective. Because soil suppressiveness was thought to be less than realized in the experimental research site (Chapter 2), this study was conducted to determine whether endospores could readily percolate vertically through the soil. The objective of this study was to determine whether endospores of *P. penetrans* could percolate vertically in soil, and whether endospores could be detected at various depths in the field.

Materials and Methods

Field Trial. The peanut field located on the Woodrow Fugate farm near Williston, Levy County, FL (Chapter 2) was used for this study. The experiment was conducted as a randomized complete block design with three treatments, replicated four times (total of 12 plots), and each replicate was composed of five subsamples per plot. The three treatments were soil from plots of bahiagrass, rhizomal peanut, and weed fallow. Soil samples were taken once a year at five depths (0-15, 15-30, 30-45, 45-60, 60-75 cm). Five soil cores per depth were removed via a bucket agar (10-cm-d) with each location about 25 m apart in the middle of each plot. The soil was classified as Arredondo fine sand [(92.5% sand, 4% silt, 3.5% clay, and <1% organic matter; pH 7.2 for the top 0-15 cm depth), (91% sand, 2% silt, 7% clay, and <1% organic matter; pH 7.4 for the 15-45 cm depth), (85% sand, 4% silt, 11% clay, and <1% organic matter; pH 7.4 for the 45-75 cm depth)]. The soil samples at each depth within each plot were combined, mixed thoroughly, placed in 10 × 15 × 20 cm, 0.002 cm thick polyethylene bags, which were placed in a cooler, and immediately taken to the laboratory for processing. All plant-parasitic nematodes were extracted by a centrifugal-flotation method (Jenkins, 1964), identified and counted.

A soil bioassay, as described below, was used to determine the presence of *P. penetrans* endospores in the soil at each soil depth each season. Soil samples were air-dried and 40 g from each were placed in a 50 ml sterile polyethylene centrifuge tube. Soil water content was adjusted to 100% field capacity (saturated) to increase the rate of endospore attachment. Then, 500, 1-to 3-day-old J2 of *M. arenaria* race 1 were added and the tubes left uncovered at room temperature. Three days later the J2 were extracted

by centrifugal-flotation method (Jenkins, 1964), and the number of endospores attached per J2 was determined with the aid of an inverted microscope. The first 20 J2 observed per sample were used.

Laboratory trial 1. The polyvinylchloride plastic tubes 55 cm long and 5 cm diameter (id) were filled up to 50 cm with an Arredondo fine sand soil by driving them into an undisturbed soil in a field located at the University of Florida Plant Science Unit, Marion County, Citra, FL. The filled tubes were removed from the field and taken to the laboratory. The top 5 cm of each tube was left unfilled to provide enough room for water to be added, and the bottom end of the tubes were covered with Nitex screen (45 μm opening) (TETKO, Elmsford, NY) to block the soil from being washed out the bottom of tubes.

Three representative tubes were used to determine soil measurement for bulk density, soil moisture, percentage organic matter, and soil texture. At the initiation of the study, bulk density and soil moisture was 1.5 g/cm^3 and 18%, respectively. The bulk density was an indication of the size of soil pore space in a given volume. The soil was classified as a Sparr fine sand, and consisted of 93% sand, 3.5% silt, 3.5% clay, and <1% organic matter; pH = 7.4.

Another three tubes were used to determine water-holding capacity at the time the experiment was initiated. Tap water at room temperature was added to tubes until it leaked from the bottom. Once water was visible at the bottom of the tube the soil was considered to be at water holding capacity. Water holding capacity was measured as 310 ml/tube. The amount of water added to each experimental tube was one-half this amount (155 ml). The treatments consisted of 0 (control), 10,000, and 20,000 endospores/g of

soil and each treatment was replicated three times in a factorial design. The experiment was conducted at room temperature. The weight of each tube was recorded before and after adding water. The two densities of *P. penetrans* (10,000 or 20,000 endospores/g of soil) were added to the top of each tube uniformly in 10 ml of water suspension. Soon after, 155 ml of water soil were added to the soil columns to bring each tube up to one-half water holding capacity. After each water application, the tubes were weighed, and water was applied to bring each tube back to one-half water holding capacity. This was repeated up to four times, i. e., one application per tube — added water once, two applications per tube— added water twice, etc. The interval between each water application was 7 to 14 days (total of 55 days for the trial).

At the end of the experiment each tube was destructively sampled by cutting it into 12.5-cm sections. Presence of endospores was determined by a bioassay of the soil collected from the middle of each 12.5-cm section (total of four sections, i.e., four depths) with a T-shaped sampling tube (2.5-cm-d). The bioassay was run for 3 days, after which the J2 were extracted by a centrifugal-flotation method (Jenkins, 1964) and the first 20 J2 were observed for the number of endospores attached per J2. Any endospores present in the water collected from the bottom of each tube were concentrated by centrifuging at 8,000*g* for 4 minutes. The water-endospore suspensions for each water application were concentrated to a 10 ml volume by discarding the excess water with a pipette. Then, 500 J2 *M. arenaria* race 1 were added and centrifuged for 2 minutes at 8,000*g*. The J2 were recovered by a centrifugal-flotation extraction method and observed for the number of endospores attached. Data were subjected to analysis of variance, and treatment means were compared by Duncan's multiple-range test.

Laboratory trial 2. The experiment reported in laboratory trial 1 was repeated after 8 months.

Pasteuria penetrans cultures. The origin of the *P. penetrans* isolate, designated P-20, was from *M. arenaria* race 1 (Neal) Chitwood, from Levy County, FL. Spores of the isolate were extracted from *M. arenaria* infected peanut (*Arachis hypogaea* L. cv. Florunner) roots grown at the original isolation site. Thirteen roots of peanut were collected from each plot at harvest and kept in a paper bag until they were processed. After 5 to 6 weeks about 10 g of dry roots from each sample were incubated for 2 days in an enzymatic maceration solution. The solution consisted of 50 ml of 0.5 M sodium acetate (pH 5.0), 50 ml of Pomaliq 2 F (Gist-Brocades, Charlotte, NC), 500 µl of 1.0 M calcium chloride, 400 ml deionized water (Charnecki, 1997). The samples were washed vigorously over stacked-sieves with 30-µm-pore openings (top) and 200-µm-pore openings (bottom). *Pasteuria penetrans* infected females, conspicuous by their opaque appearance, were picked from among the females collected from the bottom sieve with a stereo-microscope at a magnification of 20×. Five females were placed on a glass slide, covered with a cover slip, crushed and checked for the presence of *P. penetrans* endospores under the microscope at a magnification of 100×. If endospores were present they were washed into a bottle. This was repeated until sufficient endospore-filled females (about 1,000) were collected. These were macerated in a glass-tissue grinder in 1 ml deionized water, and the spore concentration was determined with a bacterial Hemacytometer counting slide (Hausser Scientific, Horsham, PA).

Results

Field trial. There were individual and combined effects of treatments, depths, and years on the percentages of J2 with endospores attached and the average number of endospores per J2 in the soil bioassay ($P \leq 0.05$) (Table 3-1). In 1999, weed fallow plots resulted in a higher percentage of J2 with endospores of *P. penetrans* attached in the top three depths than those from bahiagrass and rhizomal peanut plots ($P \leq 0.05$) (Table 3-2). However, the percentages of J2 with endospores attached varied in the deeper two depths for the same year (Table 3-2). In 1999, the average number of endospores per J2 also was greater in weed fallow plots than those in bahiagrass and rhizomal peanut plots at all depths ($P \leq 0.05$). Similar results persisted for 2000, except in the average number of endospores per J2 in the bottom two depths (Table 3-2). In 2001, the percentage of J2 with endospores attached and the average number of endospores per J2 were greater in the upper three soil depths in weed fallow plots than in the bahiagrass and rhizomal peanut plots with the exception of the 0-15 cm depth for bahiagrass ($P \leq 0.05$) (Table 3-2). In 2002, the final year of study, there was no difference in percentage of J2 with endospores attached and the average number of endospores among the treatments ($P \leq 0.05$) (Table 3-2). A similar trend was observed for J2 extracted directly from the field soil but the bioassay of soil consistently revealed a higher percentage of J2 with endospores attached and a greater average number of endospores per J2 than those in field soil samples (Tables 3-2, 3-3).

In general, the numbers of *Meloidogyne* spp. detected were relatively low among all treatments and depths (Table 3-4). Over the course of the 4-year study, there were few differences in the root-knot nematode densities among the treatments within the

Table 3-1. Analysis of variance for the individual and combined effects of depths (0-15, 15-30, 30-45, 45-60, 60-75 cm) treatments (bahiagrass, rhizomal peanut, and weed fallow) and years on the percentage of second-stage juveniles (J2) of *Meloidogyne* spp. with endospores of *Pasteuria penetrans* attached, the average number of endospores attached per J2 in a bioassay, or the average number of endospores attached per J2 in 100 cm^3 of field soil collected from each plot for four growing seasons (1999-2002).

	Percentage of J2 with endospores attached[a]		Endospores/J2[b]		
Source of variation	Bioassay[c]	Field soil	Bioassay	Field soil	*Meloidogyne* spp.
Depth (D)	*	ns	*	ns	*
Treatments (T)	*	*	*	*	*
Years (Y)	*	*	*	ns	*
D×T	*	*	*	*	ns
D×Y	*	ns	*	ns	*
T×Y	*	*	*	*	*

Table 3-1. Cont'd.

D×T×Y	*	ns	*	*	*

[a]Data transformed with $\log_{10}(x+1)$.

[b]Data transformed with arcsin ($\sqrt{x}$) before analysis.

[c]Forty grams of air-dried soil was placed in a 50 ml-size sterile polyethylene centrifuge tube. Five-hundreds, 1 to 3 days old *Meloidogyne arenaria* race 1 J2 were added. Three days later, J2 were extracted by a centrifugal-flotation method and the number of endospores attached per J2 was counted based on the first 20 J2 observed per sample.

ns = nonsignificant at $P \leq 0.05$.

Table 3-2. The percentage of second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 with *Pasteuria penetrans* endospore attached, and the average number of endospores attached per J2 (as determined from a bioassay[a] conducted on the soil) at five soil depths taken at harvest for each peanut season 1999-2002 following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

	Soil depths (cm)					Soil depths (cm)				
	0-15	15-30	30-45	45-60	60-75	0-15	15-30	30-45	45-60	60-75
Treatments[b]	Percentage of J2 with endospores attached[c]					Endospores/J2[d]				
					1999					
Bahiagrass	30 b	20 b	15 b	10 a	5 b	0.8 b	0.5 b	1.8 a	0.8 b	0.5 b
Rhizomal peanut	10 b	13 b	15 b	10 a	32 a	0.5 b	1.0 b	0.8 b	0.8 b	0.5 b
Weed fallow	75 a	57 a	63 a	32 a	35 a	2.8 a	2.5 a	2.0 a	1.5 a	3.0 a
					2000					
Bahiagrass	26 b	5 b	14 b	13 a	7 a	1.2 b	0.1 b	0.3 b	0.7 a	0.2 a
Rhizomal peanut	9 b	6 b	9 b	5 a	11 a	0.2 b	1.5 b	0.2 b	0.1 a	0.2 a
Weed fallow	66 a	43 a	45 a	30 a	14 a	5.6 a	4.8 a	1.9 a	0.3 a	0.3 a

Table 3-2. Cont'd.

					2001					
Bahiagrass	56 ab	25 b	25 b	15 b	0.3 a	3.8 ab	1.4 b	0.7 b	0.4 b	0.3 a
Rhizomal peanut	19 b	16 b	18 b	18 ab	0.4 a	1.8 b	0.5 b	0.8 b	0.5 b	0.4 a
Weed fallow	76 a	83 a	66 a	46 a	1.0 a	35.5 a	18.5 a	7.0 a	2.8 a	1.0 a
					2002					
Bahiagrass	66 a	44 a	14 a	30 a	11 a	15.1 a	2.1 b	1.6 a	1.4 a	0.3 a
Rhizomal peanut	69 a	49 a	16 a	16 a	3 a	6.3 a	2.9 ab	0.4 a	0.5 a	0.2 a
Weed fallow	80 a	83 a	44 a	45 a	19 a	32.3 a	16.6 a	3.8 a	5.4 a	1.1 a

[a]Forty grams of air-dried soil from each plot was placed in a 50 ml-size sterile polyethylene centrifuge tube. Five-hundreds of 1-to-3-day old J2 of *Meloidogyne arenaria* race 1 were added. Three days later, J2 were extracted by a centrifugal-flotation method and the number of endospores attached per J2 was counted based on the first 20 J2 observed per sample.

[b]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer.

Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

[c]Data were transformed with arcsin ($\sqrt{x}$) before analysis, but untransformed arithmetic means are presented.

Table 3-2. Cont'd.

[d]Data were transformed with $\log_{10}(x+1)$ before analysis, but untransformed arithmetic means are presented.

Data are means of four replications with 20 J2 per replication. Actual numbers are presented. Means within a column followed by the same letter are not different according to Duncan's multiple-range test ($P \leq 0.05$).

Table 3-3. The percentage of second-stage juveniles (J2) of *Meloidogyne* spp.[a] with *Pasteuria penetrans* endospore attached and the average number of endospores attached per J2 (in soil collected at five depths) at peanut harvest for the years 1999 to 2002 following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

	Soil depths (cm)					Soil depths (cm)				
	0-15	15-30	30-45	45-60	60-75	0-15	15-30	30-45	45-60	60-75
Treatments[b]	Percentage of J2 with endospores attached[c]					Endospores/J2[d]				
					1999					
Bahiagrass	0 a	10 a	5 a	15 a	5 a	0.0 a	0.3 a	0.2 a	0.4 a	0.1 a
Rhizomal peanut	0 a	5 a	5 a	0 b	5 a	0.0 a	0.1 a	0.1 a	0.0 a	0.2 a
Weed fallow	5 a	10 a	10 a	15 a	10 a	0.1 a	0.4 a	0.4 a	0.4 a	0.1 a
					2000					
Bahiagrass	5 b	5 b	9 a	3 b	6 a	0.3 a	0.2 a	0.5 a	0.1 a	0.1 a
Rhizomal peanut	5 b	6 b	3 a	6 ab	4 a	0.2 a	0.2 a	0.1 a	0.2 a	0.3 a
Weed fallow	15 a	15 a	3 a	10 a	8 a	0.5 a	0.4 a	0.1 a	0.4 a	0.1 a

Table 3-3. Cont'd.

					2001					
Bahiagrass	56 ab	25 b	25 b	15 b	11 a	1.0 b	0.2 b	0.2 b	0.2 b	0.2 a
Rhizomal peanut	18 b	16 b	18 b	18 ab	11 a	0.3 b	0.2 b	0.1 b	0.3 b	0.1 a
Weed fallow	75 a	83 a	66 a	46 a	35 a	3.2 a	5.0 a	5.1 a	1.8 a	0.1 a
					2002					
Bahiagrass	38 b	13 b	4 b	5 a	5 a	0.8 b	0.1 a	0.1 a	0.1 a	0.1 a
Rhizomal peanut	16 b	10 b	14 b	10 a	5 a	0.5 b	0.1 a	0.2 a	0.2 a	0.1 a
Weed fallow	82 a	59 a	48 a	34 a	15 b	4.7 a	4.5 a	3.5 a	3.5 a	0.3 a

[a]*Meloidogyne arenaria* race 1 is the dominant species infecting peanut at this site, however, *M. javanica* also was discovered infecting peanut in this field in the autumn of 2001 (see Chapter 5).

[b]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer. Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

. Table 3-3. Cont'd

[c]Data were transformed with arcsin ($\sqrt{x}$) before analysis, but untransformed arithmetic means are presented.

[d]Data were transformed with $\log_{10}(x+1)$ before analysis, but untransformed arithmetic means are presented.

Data are means of four replications with 20 J2 per replication. Actual numbers are presented. Means within a column followed by the same letter are not different according to Duncan's multiple-range test ($P \leq 0.05$).

Table 3-4. Number of second-stage juveniles (J2) of *Meloidogyne* spp.[a] at five soil depths at taken at harvest for each peanut season 1999-2002 following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

	Soil depths (cm)					Soil depths (cm)				
Treatments[b]	0-15	15-30	30-45	45-60	60-75	0-15	15-30	30-45	45-60	60-75
			1999					**2000**		
Bahiagrass	40 a	87 a	36 a	42 ab	43 a	1 a	1 a	0 a	0 a	0 a
Rhizomal peanut	0 b	0 b	11 a	1 b	9 a	0 a	0 a	0 a	0 a	0 a
Weed fallow	17 a	71 a	49 a	72 a	65 a	4 a	7 a	6 a	11 a	5 a
			2001					**2002**		
Bahiagrass	52 a	125 a	29 a	50 a	181 a	22 a	68 a	38 a	11 a	26 a
Rhizomal peanut	90 a	126 a	32 a	82 a	98 a	20 a	42 ab	38 a	50 a	95 a
Weed fallow	24 a	112 a	63 a	64 a	194 a	18 a	11 b	16 a	17 a	51 a

[a]*Meloidogyne arenaria* race 1 is the dominant species infecting peanut at this site, however, *M. javanica* also was discovered infecting peanut in this field in the autumn of 2001 (see Chapter 5).

Table 3-4. Cont'd.

[b]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer. Glyphosate herbicide was sprayed over the rhizomal peanut in the summer of 1991 to kill the plants. Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

Data are means of four replications with 20 J2 per replication and were transformed with $\log_{10}(x+1)$ before analysis. Actual numbers are presented. Means within a column in the same year followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

depths sampled (Table 3-4). The highest density of root-knot nematode recorded was 194/100 cm^3 in weed fallow plots in 2001, at the deepest depth, 60-75 cm.

Laboratory trials. The individual and interaction effect of soil depths, inoculum levels, number of water applications, and trials had a difference on the number of endospores detected from the soil except for the interaction effect of depth and trials, and trials alone ($P \leq 0.05$) (Table 3-5). Also the individual and interaction effects of both the number of water applications and the inoculum levels had an effect on the number of endospores detected in the water collected from the bottom of tubes (Table 3-5) ($P \leq 0.05$). There was no difference in number of endospores in the water among the trials (Table 3-5) ($P \leq 0.05$).

In trials 1 and 2, *P. penetrans* endospores were observed to percolate through the soil with water (Table 3-6). After one application of water, some endospores were detected as deep as 25-37.5 cm in both the first and second trials. Endospores were present at the greatest depth, 37.5-50 cm, after the third application of water in both trials. The number of endospores of *P. penetrans* detected among soil depths varied for both trials (Table 3-6). The number of water applications in the same inoculum density affected the number of endospores detected at various depths ($P \leq 0.05$) (Table 3-5). In trial 1, at 25-37.5 cm depth, the application of water three and four times moved a greater number of endospores than one or two water applications at the 20,000 inoculum density ($P \leq 0.05$) (Table 3-6). In the same trial, at 37.5-50 cm depth, four water applications had a greater number of endospores than one to three water applications at the 20,000 inoculum density ($P \leq 0.05$) (Table 3-6). In trial two, at 37.5-50 cm depth the three and

Table 3-5. Analysis of variance for the individual and combined effects of soil depths, inoculum densities, number of water applications, and number of trials on the endospore numbers observed on second-stage juveniles (J2) of *Meloidogyne arenaria* race 1, based on soil and water bioassays.

Source of variation	Endospores/J2[a] (soil)	Endospores/J2[b] (water)
Soil depth (D)	*	-
Inoculum density (I)	*	*
Number of water applications (A)	*	*
Trials (T)	ns	ns
D × I	*	-
D × A	*	-
D × T	ns	-
I × A	*	*
I × T	*	*
A × T	*	*
D × I × T	*	_
D × A × T	*	_
A × I × D	*	_
I × A × T	*	*
D × I × A ×T	*	_

- = No statistical analysis conducted.

[a]Based on first 20 J2 observed in the soil bioassay.

Table 3-5. Cont'd.

[b]Based on first 20 J2 of bioassay of water that collected from the bottom of each tube.

Table 3-6. Percolation of two densities of *Pasteuria penetrans* endospores through PVC tubes filled with field soil following one to four applications of water applied to each tube over 55 days.

Soil depth (cm)	Endospores/J2[a]							
	One application[b]		Two applications		Three applications		Four applications	
	Inoculum density[c]		Inoculum density		Inoculum density		Inoculum density	
	10K	20K	10K	20K	10K	20K	10K	20K
				Trial one				
0-12.5	12.5 Aa	18.2 Aa	10.1 Aa	15.3 Aa	8.5 Aa	10.8 Aa	8.7 Aa	10.5 Aa
12.5-25.0	2.0 Ab	6.3 Ab	1.5 Ab	3.0 Ab	3.2 Ab	0.5 Ac	4.9 Ab	1.5 Ac
25.0-37.5	1.8 Ab	0.1 Bc	2.0 Ab	0.5 Bc	3.0 Ab	3.1 Ab	3.3 Ac	3.5 Ab
37.5-50.0	0.0 Bc	0.0 Bc	0.0 Bc	0.0 Bc	1.2 Abc	0.5 Bc	0.8 ABd	1.8 Ac
				Trial two				
0-12.5	9.3 Aa	14.8 Aa	5.7 Aa	12.8 Aa	6.0 Aa	8.4 Aa	7.7 Aa	7.5 Aa
12.5-25.0	8.2 Aa	3.1 Ab	4.4 Aa	3.8 Ab	3.8 Ab	5.2 Ab	4.0 Ab	3.5 Ab
25.0-37.5	0.0 Bb	0.7 Bc	0.5 ABb	3.0 Ab	1.0 ABc	1.8 ABc	1.3 Ac	3.5 bA

Table 3-6. Cont'd.

37.5-50.0	0.0 Ab	0.0 Bc	0.0 Ab	0.0 Bc	0.7 Ac	2.1 Ac	1.1 Ac	2.0 Ab

[a]Five-hundered second-stage juveniles of *Meloidogyne arenaria* race 1 were used to assay endospores of *Pasteuria penetrans* in each sample.

[b]One-hundered and fifty-five milliliters of water were added to each tube per water application.

[c]A = 10,000 endospores/g of soil. B = 20,000 endospores/g of soil.

Data are means of three replications with 20 J2 per replication. Means within a column in the same trial (lower case), and within a row in the same inoculum density (upper case) followed by the same letter are not different according to Duncan's multiple-range test ($P \leq 0.05$).

four water application treatments supported a greater number of endospores than the one or two water application treatments at the 20,000 inoculum density ($P \leq 0.05$) (Table 3-6). The water application affected the number of endospores detected in the water collected from the bottom of the tubes ($P \leq 0.05$) (Table 3-5). The number of endospores detected in the water collected from the bottom of the tubes were 0, 0, 0, and 0.8 for 0-12.5, 12.5-25, 25-37.5 and 37.5-50 cm depths, respectively at10,000 inoculum level. The numbers of endospores detected in the water collected from the bottom of the tubes were 0, 0, 1, and 7.8 for 0-12.5, 12.5-25, 25-37.5 and 37.5-50 cm depths, respectively at 20,000 inoculum level.

Discussion

These studies indicate that *P. penetrans* endospores can move downward more than 75 cm in field soil. This may correspond to a decrease in the numbers of endospores over the years in the top 15 to 20 cm of soil if they are not being continuously amplified over time. Second-stage juveniles of root-knot nematodes with endospores attached were found down to 122 cm deep in a sandy field soil in Florida (Dickson et al., 1994). Thus, it is believed that *Pasteuria* spp. becomes widely dispersed through the soil profile naturally, at least in sandy soils. Downward movement with percolating water may be restricted by the characteristics of the soil such as small-pore spacing, higher clay content, organic matter, and (or) other unknown factors (Dickson et al., 1994; Mateille et al., 2002). The soil used in the laboratory trials had 93% sand, which suggests that the endospores could move readily in the pore spaces between the sand particles.

There was outcroppings of clay pockets across the field. These areas were variable in size, thereby leading to variation in the soil texture. Although not mapped, the

existence of clay probably affected both root-knot nematode distribution and endospore densities across the field. Effects of clay soils on nematode communities have been reported previously (McSorley and Frederick, 2002). Examination of the soil samples that were taken from the site at different depths also revealed that root-knot nematodes could be found down to 75 cm deep. The numbers of root-knot nematodes was low in all plots in all depths in 2000 because of the drought conditions at the field at the time of soil sampling.

Some endospores of *P. penetrans* P-20 isolate were found more than 37.5 cm deep in the laboratory experiment after three or four applications of water. This corroborates the results of the study by Oostendorp et al. (1990), who reported that in a small vertical soil-filled chamber, some endospores of P-20 isolate moved downward as deep as 3.2 cm after a single application of 5.8 ml of water. After 3 days, they detected endospores at the greatest distance of 6.4 cm from the surface. They reported no endospores in the water collected from the bottom of tubes. However, in our study with the water bioassay, we found endospores attached to J2 in the bottom of the tubes (55 cm deep). This result suggests that some endospores could be washed down by rainfall in the field, however, most of the endospores stayed in the top layer of the soil.

CHAPTER 4

PERSISTENCE AND SUPPRESSIVENESS OF *PASTEURIA PENETRANS* TO *MELOIDOGYNE ARENARIA* RACE 1 IN A PEANUT FIELD FOLLOWING 9 YEARS OF BAHIAGRASS, RHIZOMAL PEANUT, WEED FALLOW, AND 4 YEARS OF CONTINUOUS PEANUT.

Introduction

Suppressive soils may be defined as those in which disease development in a susceptible host is suppressed even though the pathogen is present (Huber and Schneider, 1982). Suppressiveness of soil to plant pathogens may develop naturally as an inherent characteristic of their physical, chemical, and (or) biological structure. Build up of biological control agents (antagonists) in response to a high pathogen population can result in soils becoming suppressive (Baker and Cook, 1984). Some agronomic practices that change microflora may induce soil suppressiveness especially when susceptible plants are grown in sequence (Baker and Cook, 1984).

Pasteuria penetrans (Thorne) Sayre & Starr is an obligate, endospore-forming bacterium that has been shown to effectively suppress root-knot nematodes populations in field and microplots experiments (Brown and Smart, 1985; Chen et al., 1996; Freitas et al., 2000c; Oostendorp et al., 1991; Stirling 1984; Weibelzahl-Fulton et al., 1996). The organism's extended survival in soil, host specificity, tolerance to heat, desiccation, and some nematicides make *P. penetrans* one of the more promising environmentally friendly biological control agents for plant-parasitic nematodes (Chen and Dickson, 1998; Stirling, 1991; Bekal et al., 2000). In most studies, suppressiveness of soils to

Meloidogyne spp. was observed after several years of crop production (Bird and Brisbane 1988; Chen et al., 1994; Mankau, 1980; Minton and Sayre, 1989). The organism was reported (Dickson et al., 1994) to cause suppressiveness of *Meloidogyne arenaria* race 1 in a peanut field located on the Woodrow Fugate farm near Williston, Levy County, FL. This site had been used for peanut nematode research for several years beginning in 1970.

Because of the suppressive nature of the soil at this site, a study of long-term survival of *M. arenaria* race 1 and *P. penetrans* was undertaken in the summer of 1991. A randomized complete block design was established with three treatments and replicated ten times. The three treatments were two *M. arenaria* race 1 nonhost crops (bahiagrass *Paspalum notatum* Flugge cv. Pensacola var. Tifton 9), rhizomal peanut (*Arachis glabrata* Benth. cv. Florigraze), and weed fallow. All plots were planted with rye (*Secale cereale* L. cv. Wrens Abruzzi) during the autumn-winter season as a cover crop. Bahiagrass and weed fallow are not hosts of *M. arenaria* race 1. After 9 years, bahiagrass and weed fallow plots were destroyed by plowing and disking. The rhizomal peanut had developed such a deep and thick thatch that the site could not be plowed and disked. Therefore, the herbicide, glyphosate, was used to kill the plants prior to plowing and disking. Because of having to use the herbicide, planting peanut was delayed for one season. Thus peanut was grown on this site only three consecutive years. Peanut *Arachis hypogaea* L. was planted and grown four consecutive years.

Autoclaving was used to kill all agents in soil as previously reported (Chen et al., 1995;Weibelzahl-Fulton et al., 1996), thereby allowing root-knot nematodes to be infective. Microwaving of soil was used as an improved method for reducing fungal

antagonists. Earlier observations have shown that microwaving soil is an effective method to reduce possible fungal antagonists (Chen et al., 1995; Ferris, 1984; Weibelzahl-Fulton et al., 1996). Whereas air-dried and untreated soils would contain antagonists that would be suppressive to *M. arenaria* race 1.

The objective of the experiment was to determine if *P. penetrans* had persisted and increased to the extent that populations of *M. arenaria* race 1 would be suppressed.

Materials and Methods

One hundred and twenty liters of soil was collected from each of three plots (bahiagrass, rhizomal peanut, and weed fallow) by taking 20 soil cores randomly. The soil from each site was mixed thoroughly and divided in four sub-samples of 30 liters each for four treatments. The treatments were autoclaving, microwaving, air-drying, and untreated. Each treatment was replicated five times. Moisture of the soil was 18% when the soil was prepared for the treatments.

Autoclaving. Previously sterilized clay pots (15-cm-diameter) were filled with about 1 liter of soil, and the pots covered with aluminum foil and autoclaved for 99 minutes at 55 kPa. After the pots cooled to room temperature they were autoclaved again (Mulder, 1979).

Microwaving. The 30 liters of soil were microwaved 1 kg at a time. The 1 kg of soil was placed in even layers in plastic bags, which were left open, and heated in a microwave oven for 3 minutes (Ferris, 1984) at full power (625 W, 2,450 MHz), which increased the soil temperature to about 75 °C.

Air-drying. The soil was placed in 4-5 cm thick layers on a polyethylene sheet on a green house bench for 2 weeks. The soil was stored in a greenhouse until used.

Untreated. The untreated soil was stored in a cool room (10 °C) until used.

One hundred milliliters of water were added to the air-dried soil and 50 ml to all other soil treatments. Then, 2,500 J2 of *M. arenaria* race 1 were dispensed in 8 ml of water to each of four holes (2 ml of nematode suspension each) about 5 cm deep in each pot. After 1 week, one 4-week-old tomato seedling, *Lycopersicon esculentum* Mill. cv. Rutgers, was transplanted into each pot. The pots were kept in a growth room at 28 to 32 °C and 16 hours of light. Every 10 to 14 days, insects were managed by using insecticidal soap and every week the plants were fertilized by Peters Professional 20-20-20 (N, P_2O_5, and K_2O, respectively) (Scotts-Sierra Horticultural Products, Maryville, OH) at a rate of 3 g/liter of water. After 57 days the plant stems were cut off at ground level and discarded. The plant roots were removed, washed free of soil, and stored in a cool room in plastic bags at 10 °C for processing. The roots were examined over a 2-day period, and the number of root galls counted. Eggs were counted following extraction with 0.5% sodium hypochlorite (Hussey and Barker, 1973).

Nematode population. *Meloidogyne arenaria* race 1 originated from peanut grown at the University of Florida, Green Acres Agronomy Farm, Alachua County, FL. The nematode was cultured in a greenhouse on Rutgers tomato. Inoculum of *M. arenaria* race 1 was collected from galled roots by dissolving the gelatinous matrices with 0.5% sodium hypochlorite (Hussey and Barker, 1973) and catching eggs on a sieve with 25 µm-pore openings. Second-stage juveniles were obtained by hatching the eggs on a modified Baermann funnel (Rodríguez-Kábana and Pope, 1981). They were used within 1 to 3 days after they hatched.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC) and mean treatment differences were separated and compared using Duncan's multiple-range test.

Results and Discussion

In the soil from the weed fallow and rhizomal peanut sites, root galling was significantly greater in the autoclaved and microwaved treatments than in the air-dried and untreated treatments. In soil from the bahiagrass site, root galling was significantly greater in the autoclaved, microwaved, and untreated treatments than in the air-dried treatments.

Regardless of the soil source (bahiagrass, rhizomal peanut, and weed fallow plots), peanut grown in the autoclaved soil treatments contained significantly more eggs than any other treatments ($P \leq 0.05$) (Table 4-1). The number of eggs was not significantly different in the microwaved and untreated soil for any treatment. For the air-dried soil, there were no eggs from the weed fallow and bahiagrass sites, but the number of eggs from the rhizomal peanut site was not significantly different from the numbers from microwaved or untreated soil. There were no differences in the number of eggs between microwaved and untreated soil among all treatments ($P \leq 0.05$). In two of the treatment soils (weed fallow and bahiagrass) there were no eggs recovered in the air-dried treatment, and only 10 eggs were recovered in the rhizomal peanut treatment (Table 4-1).

The main effects of soil source, treatment, and inoculation densities varied in the study. Soil sources did not affect the number of eggs and galls when both inoculum levels were included in the analysis (Table 4-2). The soil treatments and inoculation

Table 4-1. Number of eggs and gall percentages on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) grown in *Pasteuria penetrans* infested soil that was treated by autoclaving, microwaving, air-drying, and untreated, after inoculation with 0 or 2,500 second-stage juveniles of *Meloidogyne arenaria* race 1. The soil was collected from peanut plots following 9 years of growing bahiagrass, rhizomal peanut, weed fallow, and then 4 years of continuous peanut.

Soil source[a]	Soil treatment[b]	% Galling[c]		Number of eggs[d]	
		Inoculation density		Inoculation density	
		0	2,500	0	2,500
Weed fallow	Autoclaved	0 b	60 a	0 a	1,150 a
	Microwaved	0 b	55 a	0 a	180 b
	Air-dried	0 b	17 b	0 a	0 c
	Untreated	4 a	20 b	0 a	90 b
Bahiagrass	Autoclaved	2 a	50 a	0 a	11,910 a
	Microwaved	5 a	45 a	0 a	260 b
	Air-dried	0 b	15 b	0 a	0 c
	Untreated	0 b	40 a	0 a	120 b
Rhizomal peanut	Autoclaved	0 b	60 a	0 a	6,640 a
	Microwaved	0 b	40 a	0 a	110 b
	Air-dried	0 b	10 b	0 a	10 b
	Untreated	0 b	15 b	0 a	60 b

[a]Soils were taken from three treatments (bahiagrass, rhizomal peanut and weed fallow), which were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer. Bahiagrass and weed fallow plots were planted with peanut during summers of

Table 4-1. Cont'd.
1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

[b]Autoclaved twice for 99 minutes each at 55 kPa, microwaved at full power for 3 minutes/kg of soil, air-dried for 2 weeks on a greenhouse bench, and stored in a cool room at 10 °C for until it was used (untreated).

[c]Root galling was determined at harvest based on a scale of 0 to 100 (0 = no galls 100 = 100% of root system galled).

[d]Eggs were collected from galled roots by dissolving the gelatinous matrices with 0.5% sodium hypochlorite and catching individual eggs on a sieve with 25 µm-pore openings.

Data are means of five replications. Means within a column in the same soil source followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

Table 4-2. Analysis of variance table for the effect of soil sources (soil taken from bahiagrass, rhizomal peanut, and weed fallow plots), soil treatments (autoclaved, microwaved, air-dried, and untreated), and based on *Meloidogyne arenaria* race 1 inoculum densities of 0 and 2,500 second-stage juveniles or 2,500 J2 alone on number of galls and nematode reproduction following 9 years of growing bahiagrass, rhizomal peanut, weed fallow, and 4 years of continuous peanut.

Source of variation	Number of galls	Number of eggs
Two inoculum levels: 0 and 2,500 J2		
Soil source (S)	ns	ns
Soil treatment (T)	**	**
Inoculation density (I)	**	**
S × T	*	**
S × I	**	**
I × T	**	**
I × T × S	**	**
One inoculum level: 2,500 J2		
Soil source (S)	ns	**
Soil treatment (T)	**	**
S × T	**	**

ns = nonsignificant

levels together affected the number of galls and eggs if the inoculum level of 0 nematode is excluded because it produces only negative data. The effect of soil sources differed in the number of eggs but not with the number of galls when only the inoculum level of 2,500 J2 was considered ($P \leq 0.01$). There was a combined effect of soil sources and treatment on the number of galls and the number of eggs ($P \leq 0.01$) (Table 4-2). However, no difference was observed in the untreated soil.

More studies have been conducted on fungal antagonists of nematodes causing soil suppressiveness than bacterial agents of nematodes (Stirling and Mankau, 1978; 1979; Kerry, 1982; Westphal and Becker, 2000). Of the many nematode antagonists, *P. penetrans* has attracted a great interest over the past 3 decades. (Chen et al., 1997; Dickson, 1994; Dickson et al., 1991; Freitas et al., 2000c). Fecundity of root-knot nematodes may be reduced up to 94% in soils where *P. penetrans* was applied (Gowen et al., 1989). Dickson et al. (1991) reported that a root-knot nematode infected site that had a heavy, damaging population density eventually became highly suppressive by *P. penetrans*. A suppressive soil test revealed a suppressive agent that was believed to be *P. penetrans* in the air-dried or untreated soil (Dickson, 1994). However, in this case formalin was used as a method to reduce possible fungal antagonists.

In this current study, microwaving was used as an improved method for reducing fungal antagonists. This supported earlier observations that microwaving is an effective method to reduce possible fungal antagonists (Chen et al., 1995; Ferris, 1984; Weibelzahl-Fulton et al., 1996). The time required for effective treatment with microwaving varies with soil moisture, and output of the microwave oven (Ferris, 1984). In this study, in the microwave treatment, numbers of galls were high but numbers of the

eggs were relatively low. This indicates that fecundity was reduced because females inducing galls were filled with spores of *P. penetrans*. The fact that microwave treatment had a higher incidence of galling than occurred in air-dried or untreated soil indicates that the number of endospores in soil may have been reduced by that treatment (Weibelzahl-Fulton et al., 1996). In the air-dried and untreated soils, except for the untreated soil from bahiagrass, there was a reduction in galling. When *P. penetrans* is at high densities and more than 1 to 5 endospores attach per J2, mobility is greatly reduced (Davies et al., 1991). While for each treatment of soil from bahiagrass, rhizomal peanut, and weed fallow there remained a suppressive level of *P. penetrans*, soil from weed fallow retained a higher density of *P. penetrans* than either bahiagrass or rhizomal peanut. This suggests that for *P. penetrans* to remain at maximum suppressive levels, some root-knot nematode infection and egg production must occur as might be provided on weed hosts. Microwaving the soil aided in separating suppressiveness caused by fungal agents and *P. penetrans*. Microwaving reduced the fungal antagonists with minor effects on the *P. penetrans*, thereby allowing relatively high numbers of galls but reduction in the number of eggs. Gowen et al., (1989) reported that *P. penetrans*-infected females are able to form giant cells and galls, but these females are unable to produce eggs because their fecundity is reduced by *P. penetrans* infection.

We assume that the autoclaving killed all agents as previously reported (Chen et al., 1995;Weibelzahl-Fulton et al., 1996), thereby allowing root-knot nematodes to be infective, whereas air-drying and untreated soils contained antagonists that were suppressive to *M. arenaria* race 1. Data from the bioassays (Chapter 2, Table 2-2) showed that following 9 years of bahiagrass, rhizomal peanut and weed fallowing low

numbers of *P. penetrans* endospores were present, especially in bahiagrass and rhizomal peanut plots. After 4 years of continuous peanut in the field, *P. penetrans* was again found to be at a suppressive level in the soil suppressive test. However, there was no conclusive evidence that the same degree of suppressiveness as that reported in 1994 (Dickson et al., 1994) was still in the field even through relatively high numbers of endospores were present.

With the complicating factor that *M. javanica* was found to infect peanut at the site in the early autumn of 2001 (Cetintas and Dickson, 2003; Cetintas et.al., 2003; Lima et al., 2002; Chapter 5), it was not possible to separate the galling induced by the two nematodes in the field. The level of infection on peanut in 2002 by *M. javanica* is not known, but the frequency of *M. javanica* among the root-knot nematode females detected from peanut roots taken at harvest ranged from 5% to 29% (Chapter 5, Table 5-2). It is not known whether this *P. penetrans* isolate attaches to and infects *M. javanica*. It is possible that *M. arenaria* race 1 is being suppressed by *P. penetrans* at the Williston site but, if not, *M. javanica* may be increasing to a dominant level.

The level of suppressiveness in these suppressive soil tests is clearly higher than the level we observed in the field (Chapter 2). It is possible that *M. javanica* is a complicating factor that is resulting in a high level of galling on peanut. These results suggest that *P. penetrans* may still be a major factor in suppressing the population density of *M arenaria* race 1, even though the peanut yields and the amount of galling on the roots do not reflect a highly suppressive soil to root-knot nematodes (Chapter 2, Table 2-5).

CHAPTER 5
MELOIDOGYNE JAVANICA ON PEANUT IN FLORIDA

Introduction

Several species of root-knot nematodes are pathogens of peanut, *Arachis hypogaea* L. Worldwide, *Meloidogyne arenaria* (Neal) Chitwood (peanut root-knot nematode) is the most common species infecting peanut (Dickson, 1998). It is found on peanut in the southeastern United States (Ingram and Rodríguez-Kábana, 1980; Powers and Harris, 1993; Sturgeon, 1986), whereas *M. hapla* Chitwood (northern root-knot nematode) is encountered more frequently in the more northern peanut production regions of the United States (Dickson, 1998). In addition, *M. javanica* (Treub) Chitwood (Javanese root-knot nematode) also has been reported to infect peanut in the United States and as well as other parts of the world. This nematode was first reported on peanut in Zimbabwe (Martin, 1958). Eleven years later, Minton et al. (1969) reported *M. javanica* on peanut in Georgia, USA. Later, the nematode was reported in Texas, USA (Tomaszewski et al., 1994). Other reports of *M. javanica* on peanut are from Africa (Ibrahim and El-Saedy, 1976; Rammah and Hirschmann, 1990; Tomaszewski et al., 1994), India (Patel et al., 1988; Sakhuja and Sethi, 1985), and Brazil (Lordello and Gerin, 1981).

In 2001, a single root-knot nematode infected peanut plant collected from a commercial production field in Levy County, FL was brought to the laboratory for species determination. Biochemical analysis of the females extracted from the roots of

this plant showed a typical esterase J3 phenotype for *M. javanica* (Lima et al., 2002). A follow-up was made 1 week later when roots were collected from volunteer peanut plants in an experimental research site located on the same farm. This field had just been harvested and the only remaining peanut plants were volunteers in the borders. These samples revealed a mixture of *M. javanica* and *M. arenaria*. The objective of this study was to confirm the occurrence of *M. javanica* on peanut in Florida based on these preliminary observations and, to determine the ratio of *M. javanica* to *M. arenaria* race 1 on peanut at the experimental site.

Materials and Methods

The experimental site was located in a production field on the Woodrow Fugate farm near Williston, FL, Levy County. There was a known infestation of *M. arenaria* race 1 and a population of *P. penetrans* that was specific to and suppressive for the nematode (Dickson et al., 1994). From 1991 to 1999, the site consisted of three treatments, bahiagrass (*Paspalum notatum* Flugge cv. Tifton 9), rhizomal peanut (*Arachis glabrata* Benth. cv. Florigraze), and weed fallow plots, each replicated 10 times in a randomized complete block design.

In 1999, the bahiagrass and weed fallow plots were plowed and disked, and the rhizomal peanut plots were treated with glyphosate. The latter was plowed and disked along with all other plots in the winter of 2000. In 1999, the bahiagrass and weed fallow plots were planted with peanut (*Arachis hypogaea* L. cv. Florunner). Peanut cv. Southern Runner was planted in 2001, and cv. Georgia Green was planted in 2001and 2002. Each plot consisted of 10 rows of peanut, each 38 m long. The row spacing was 90 cm.

Meloidogyne javanica on peanut based on soil samples. Soil samples were taken from each plot on 4 February 2002. Twelve cores (2.5 cm-diam., 20 cm-deep) were taken per plot in a zig-zag pattern with a cone-shaped sampling tube. The soil from each plot was mixed thoroughly and divided in to three portions, each of which was placed in a 12-cm diameter clay pot. Either tomato (*Lycopersicon esculentum* cv. Rutgers) or pepper (*Capsicum annuum* L. cv. California Wonder) seedlings were transplanted into 30 pots each. The remaining 30 pots were seeded with peanut (*Arachis hypogaea* L. cv. Florunner). The plants were grown in a greenhouse for 105 days, after which the plants were removed and the roots washed and examined for galling. Thirteen females were extracted from each peanut plant, each from a single gall. The females were preserved individually in 10 µl of an extraction buffer (55% deionized water, 12% 0.5 M Tris-HCl, pH 6.8, 30% glycerol, 2% of 0.5% [w/v] bromophenol blue) contained in a cone-shaped microfuge tube and placed in a freezer at -5 °C.

Meloidogyne javanica on peanut roots grown in field site. In 2002, females of root-knot nematodes were extracted from 125-day-old peanut (cv. Georgia Green) roots taken from each plot. Fresh roots were collected randomly from 13 plants in each plot. One female was extracted from each plant and stored in the extraction buffer as stated above.

Meloidogyne javanica standard control. Females of a known greenhouse isolate of *M. javanica* were extracted from tomato roots and stored in a freezer in the extraction buffer reported above.

Electrophoresis, sample preparation, and loading. A Bio-Rad mini-PROTEIN II (Bio-Rad, Philadelphia, PA) electrophoresis unit was used. Before electrophoresis, the females were thawed and homogenized individually in a microhaematocrite plastic tube

in 10-µl of extraction buffer. Each sample then was loaded into a well. Each gel contained 15 wells. The standard *M. javanica* female extract was placed into wells 1 and 14. The remaining 13 wells were loaded with the protein extract from test sample females. Electrophoresis was carried out in a discontinuous buffer system with 8% acrylamide running gel, pH 8.8 and 4% acrylamide stacking gel, pH 6.8. The voltage was maintained at 80 volts for the first 15 minutes and increased to 200 volts for the remainder of the running period. Following electrophoresis, the gels were removed and placed in an enzyme reaction mixture to determine esterase and malate dehydrogenase activity (Harris and Hopkinson, 1976).

Preparation of perineal patterns. Single egg mass cultures of *M. arenaria* and *M. javanica* were prepared from peanut roots. Confirmation of these cultures was based on isozyme phenotypes and perineal patterns. Forty females each from these two cultures were extracted from roots of peanut grown in a greenhouse. Perineal patterns were prepared following procedures of Hartman and Sasser (1985). Morphological observations and photographs of perineal patterns were completed within 12 hours following slide preparation.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC) and mean treatment differences were separated and compared using Duncan's multiple-range test.

Results

Meloidogyne javanica on peanut based on soil samples. Of the plants grown in a greenhouse, tomato and peanut roots were heavily galled, but no galling was observed on the pepper roots. Tomato roots were not processed further because tomato is a known

susceptible host for the three most common root-knot nematode species, *M. arenaria*, *M. incognita*, and *M. javanica*. Biochemical analysis of the *Meloidogyne* spp. females extracted from peanut roots revealed that 5.7% of 290 individuals had a typical esterase pattern for *M. javanica* J3 phenotype (Fig. 5-1, A, B). The remaining individuals showed a typical esterase pattern for *M. arenaria* phenotype A2. There was no effect of rhizomal peanut (6.2%), bahiagrass (5.5%), or weed fallow (5.4%) on the frequency of *M. javanica* ($P > 0.05$) (Fig. 5-2).

Meloidogyne javanica on peanut roots grown in the experimental field site. Of the samples collected at harvest in the autumn of 2002 from roots of peanut grown in field soil, 29% of the 290 individuals collected showed a typical esterase pattern for *M. javanica*. The remaining individuals showed a typical esterase pattern for *M. arenaria* phenotype A2. The weed fallow plots supported the greatest percentage of *M. javanica* (41%), whereas rhizomal peanut plots had 24%, and bahiagrass plots had 23% ($P \leq 0.05$) (Fig. 5-2). The *M. javanica* esterase phenotype was identical with the known greenhouse isolate of *M. javanica*. They both had the typical three-band esterase isozyme phenotype. The malate dehydrogenase phenotype also confirmed the occurrence of *M. javanica* on peanut (Fig. 5-1, C).

Perineal patterns. The observation of perineal patterns cut from single females extracted from peanut roots revealed morphological characters that were typically for *M. javanica* and *M. arenaria* (Fig. 5-3). *M. javanica* perineal patterns had a moderately high dorsal arch and conspicuous lateral lines (Fig. 5-3, B), whereas *M. arenaria* had a low dorsal arch, irregular striae, and an absence of lateral ridges (Fig. 5-3, A).

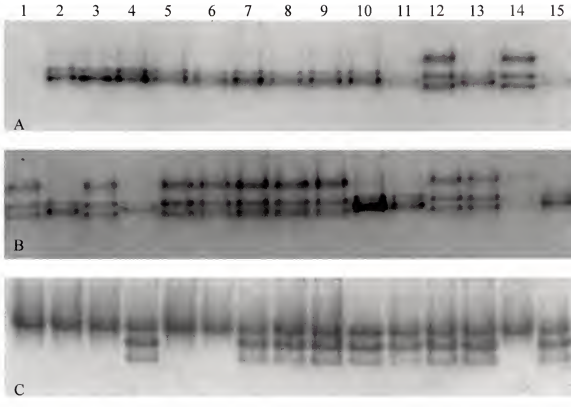

Fig. 5-1. Esterase (A, B) and malate dehydrogenase (C) (Mdh) isozymes resolved from individual root-knot nematode females following electrophoresis on a polyacrylamide slab gel. Shown are the three- and two-isozyme esterase phenotypes of esterase for *Meloidogyne javanica* (J3) and *Meloidogyne arenaria* (A2), and the one- and three- isozyme phenotypes of Mdh for *M. javanica* (N1) and *M. arenaria* (N3). A. Females were extracted from roots of 105-day-old peanut plants grown in a greenhouse in pots filled with soil taken from a peanut field near Williston, FL. Lanes 1 and 14 = *M. javanica*, standard control; lane 2-11, 13, 15 = *M. arenaria* race 1 A2 esterase phenotype; lane 12 = *M. javanica* J3 esterase phenotype. B. Females were extracted from peanut roots taken at harvest from a field near Williston, FL. Lanes 1 and 14 = *M. javanica* standard control; lanes 2, 4, 10, 11, 15 = *M. arenaria* race 1 A2 esterase phenotype; lanes 3, 5-9, 12, 13 = *M. javanica* esterase phenotypes J3. C. Females were extracted from peanut roots taken at harvest from a peanut field near Williston, FL. Lanes 1, 14 = *M. javanica* standard control, lanes 2, 3, 5, 6, *M. javanica* N1 Mdh phenotype; lanes 4, 7-13, 15 = *M. arenaria* race 1 N3 Mdh phenotype.

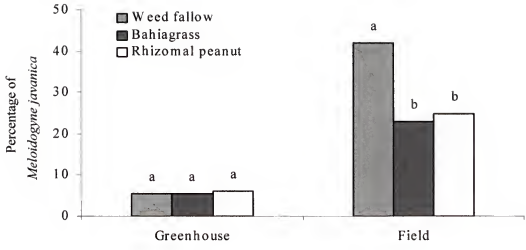

Fig. 5-2. The percentage of *Meloidogyne javanica* phenotype J3 found in peanut as determined by esterase phenotypes. Each column is an average of 10 replications (based on total of 130 females), and means with the same letter within each trial are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$). *Greenhouse:* Data are based on females extracted from roots of 105-day-old peanut grown in a greenhouse in pots filled with soil taken from a peanut field near Williston, FL. *Field:* Data are based on females extracted from peanut roots taken at harvest in 2002.

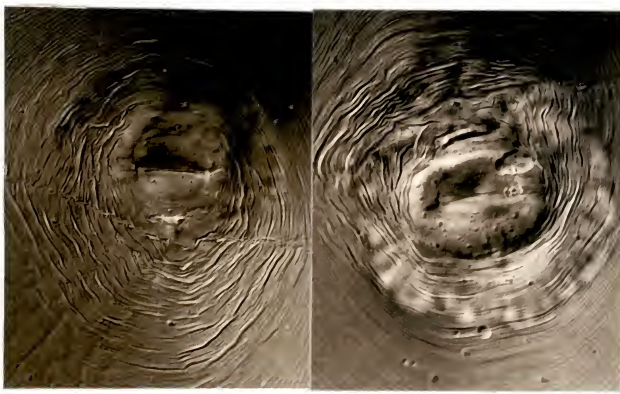

Fig. 5-3. A) Perineal pattern of *Meloidogyne javanica*. B) Perineal pattern of *Meloidogyne arenaria*. Each perineal pattern was derived from a single egg mass isolate of the two nematode species grown on peanut in a greenhouse. The high dorsal arch and lateral lines of *M. javanica* and the deep shoulders and low dorsal arch of *M. arenaria* are visible.

Discussion

This report of *M. javanica* infecting peanut in Florida confirms our earlier finding of *M. javanica* on peanut in a commercial peanut field (Lima et al., 2002). The ratio of *M. javanica* esterase phenotype to the *M. arenaria* race 1 phenotype increased from 5.7% (based on peanut plants grown in soil collected from the field) in February 2002 to 29% (based on peanut plants collected at harvest from the field) in October 2002. The greatest increase of *M. javanica* occurred in the weed fallow plots, which likely resulted from the maintenance of the nematode on weeds over the 10-year period of the experiment.

Because there was infection by *M. javanica* on peanut but none on pepper we conclude that the population is race 3 (Dickson, 1998; Rammah and Hirshmann, 1990). Despite being rare in most parts of the world (Hartman and Sasser, 1985), *M. javanica* populations capable of parasitizing peanut appear to be mostly distributed in Africa and Asia (Dickson, 1998;Tomaszewski et al., 1994), whereas their occurrence on peanut in North America appears to be relatively infrequent (Tomaszewski et al., 1994). As Minton et al. (1969) stated, however, both *M. arenaria* and *M. javanica* cause similar symptoms on peanut. This may result in the false assumption that all heavily galled peanut roots, pods, and pegs are caused by *M. arenaria*. A simple analysis of individual females by electrophoresis and staining for esterase and Mdh activity would make clear the presence of *M. javanica*. It is unclear at this time whether *M. javanica* infects peanut in other production regions in Florida.

This is the third state in the United States in which *M. javanica* has been found infecting peanut with the first and second states being Georgia and Texas, respectively. Although this study of *M. javanica* on peanut was in an experimental research field, the

nematode also was found on peanut in the grower's production field ca. 5 km from the experimental research site (Lima et al., 2002). The research site was originally designed for studying the persistence of *P. penetrans* on *M. arenaria* race 1. It is unclear at this time whether the bacterium also parasitizes *M. javanica* race 3, however when the isolate of *P. penetrans* that developed in *M. arenaria* was exposed to an *M. javanica* population only a very low rate of attachment was attained (Oostendorp, 1990). Development inside the females of *M. javanica* was not determined nor was the race of *M. javanica* used in this experiment known. This is especially interesting in that if this population of *P. penetrans* is specific and suppressive to *M. arenaria* only (Dickson et al., 1994), then we might speculate that *M. javanica* will eventually replace *M. arenaria* as the dominant species on peanut in this field.

CHAPTER 6
SUMMARY

Use of nematicides has created great controversy because of associated environmental problems. Recently, great attention has been given to alternative nematode control management tactics, such as cultural, plant resistance, and biological control with microbial agents. *Pasteuria penetrans* (Thorne) Sayre & Starr is a promising environmentally friendly biological control agent for root-knot nematodes.

The persistence and suppressiveness of *P. penetrans* in field soil, and the effects of growing root-knot nematode nonhost crops (such as rhizomal peanut and bahiagrass) compared to weed fallow (including nematode susceptible weeds) were investigated. In a 4-year study, the average number of *P. penetrans* endospores per second-stage juvenile (J2), and percentage of J2 with endospores attached were nearly undetectable in the field soil samples during the first year (1999) in the all plots. However, they increased over the 4-year period of the experiment (1999 to 2002). Average number of endospores adhering to J2 increased to a high of 5.8 in the weed fallow plots by the fall of 2001, but decreased in the fall of 2002. Weed fallow plots sustained the highest percentage of J2 with endospores attached: 87% by the fall of 2000.

In soil-depth studies for percolation and detection of *P. penetrans* endospores, both laboratory and field experiments revealed that *P. penetrans* endospores could move down through soil to depths of 75 cm. When water was applied to soil, most of the

endospores stayed on the top layer; however, but some were detected at the lowest layer of the soil (50 cm) and in the water collected from the bottom of tubes.

A test was conducted to determine suppressiveness of *P. penetrans* in a peanut field soil following 9 years of bahiagrass, rhizomal peanut, or weed fallow. All soil sources (from bahiagrass, rhizomal peanut, and weed fallow plots) still contained a suppressive level of *P. penetrans*. However, weed fallow retained a higher density of *P. penetrans* than either bahiagrass or rhizomal peanut. In this study, autoclaving presumably killed all biological agents (including *P. penetrans*), thereby allowing root-knot nematodes to increase. Air-dried and untreated soils contained antagonists that were suppressive to *M. arenaria* race 1. However, there was no conclusive evidence of the same degree of suppressiveness as reported in 1994 (Dickson et al., 1994).

Biochemical analysis of *Meloidogyne* spp. females extracted from roots of peanut collected from the experimental research site in Levy County, Florida, revealed the presence of *Meloidogyne arenaria* race 1 and *M. javanica* race 3 on peanut. The confirmation of *M. javanica* on peanut was based on esterase and malate dehydrogenase isozyme patterns resolved on polyacrylamide slab gels following electrophoresis and perineal patterns. Up to 29% of 290 individual females collected from peanut roots in the field in the autumn of 2002 showed a typical esterase J3 phenotype for *M. javanica*. This is the third report of *M. javanica* infecting peanut in the United States.

During the 4-year of study, *Pasteuria penetrans* persisted over long periods of time in the all three long-established treatments (bahiagrass, rhizomal peanut, and weed fallow) plots, but failed to suppress root galling on peanut. Maybe the reason is that *M. javanica* was discovered in the site in the third year of research. *Meloidogyne javanica* is

not known to be a host for the isolate of *P. penetrans* that infects *M. arenaria* race 1. Thus, *M. arenaria* race 1 may have been suppressed sufficiently by *P. penetrans* to reduce galling to the low levels experienced in 1991, and *M. javanica* might have been present long enough before it was detected in 2001 to have been responsible for much of the root galling.

Appendix A

CRICONEMOIDES SPP. POPULATION DENSITIES OBSERVED IN THE SOIL SAMPLES.

Table A-1. The initial (Pi), mid-season (Pm), and final (Pf) population densities of *Criconemoides* spp. based on 100 cm^3 of soil taken from peanut grown over four consecutive summer seasons (1999-2002) following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

Treatments[a]	Final population (Pf)	Initial population (Pi)	Mid-season population (Pm)
		1999	
Bahiagrass	10 b	113 a	212 a
Rhizomal peanut	13 b	71 a	46 b
Weed fallow	30 a	127 a	154 a
		2000	
Bahiagrass	62 a	12 b	22 a
Rhizomal peanut	22 b	21 ab	18 a
Weed fallow	31 b	41 a	14 a
		2001	

Bahiagrass	11 a	41 a	21 a
Rhizomal peanut	7 a	42 a	18 a

Table 2-1. Cont'd.

Weed fallow	10 a	47 a	18 a
	2002		
Bahiagrass	11 ab	16 a	61 a
Rhizomal peanut	8 b	16 a	39 a
Weed fallow	17 a	14 a	67 a
	ANOVA		
Treatments (T)	ns	ns	ns
Years (Y)	*	*	*
T×Y	*	*	*

[a]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer.

Table 2-1. Cont'd.

Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

Data are an average of 10 replications. Means within each column in the same year with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

Table A-2. Analysis of variance for the individual and combined effects of depths (0-15, 15-30, 30-45, 45-60, 60-75 cm) treatments (bahiagrass, rhizomal peanut, and weed fallow) and years on the number of *Criconemoides spp.* /100 cm^3 of field soil collected from each plot for four growing seasons (1999-2002).

Source of variation	*Criconemoides* spp.
Depth (D)	*
Treatments (T)	*
Years (Y)	*
D×T	*
D×Y	*
T×Y	*
D×T×Y	*

Data transformed with $\log_{10}(x+1)$.

Table A-3. Number of *Criconemoides* spp. at five soil depths at taken at harvest for each peanut season 1999-2002 following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

	Soil depths (cm)					Soil depths (cm)				
Treatments[a]	0-15	15-30	30-45	45-60	60-75	0-15	15-30	30-45	45-60	60-75
			1999					**2000**		
Bahiagrass	89 a	66 a	20 a	25 a	36 a	3 a	2 a	2 a	0 a	0 a
Rhizomal peanut	44 a	17 b	23 a	2 a	4 a	2 a	3 a	0 a	1 a	0 a
Weed fallow	36 a	95 a	35 a	18 a	20 a	4 a	2 a	1 a	1 a	0 a
			2001					**2002**		
Bahiagrass	18 a	9 a	5 a	2 a	4 a	99 a	40 a	6 a	2 a	1 a
Rhizomal peanut	31 a	24 a	5 a	2 a	6 a	43 a	9 a	7 a	3 a	1 a
Weed fallow	61 a	19 a	14 a	7 a	7 a	84 a	15 a	2 a	6 a	3 a

Table A-3. Cont'd.

[a]Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer of 1999, bahiagrass and weed fallow were removed for planting peanut, however the rhizomal peanut could not be removed because of heavy thatch layer. Glyphosate herbicide was sprayed over the rhizomal peanut in the summer of 1991 to kill the plants. Bahiagrass and weed fallow plots were planted with peanut during summers of 1999 to 2002, whereas rhizomal peanut plots were planted during summers of 2000 to 2002.

Data were transformed with $\log_{10}(x+1)$ before analysis. Actual numbers are presented. Means within a column in the same year followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope, and quality, as a dissertation for the degree of Doctor of Philosophy.

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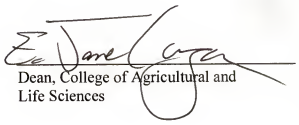

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